



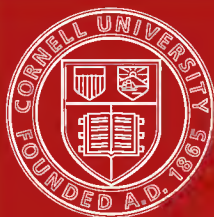
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HAND-BOOK  
OF  
CHEMICAL .  
**Physiology and Pathology,**

WITH LECTURES UPON  
NORMAL AND ABNORMAL URINE,

BY  
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OF THE DOMESTIC FOWL," "CHARTS FOR THE ANALYSIS OF ABNOR-  
MAL URINE," "A NEW METHOD OF DETECTING AND  
SEPARATING ARSENIC, ANTIMONY AND  
OTHER POISONS," ETC.

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THIRD EDITION, REVISED AND ENLARGED.


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ANN ARBOR:  
ANN ARBOR PRINTING AND PUBLISHING COMPANY.  
1880.

Entered according to Act of Congress, in the year 1880,

By VICTOR C. VAUGHAN, M. D., PH. D.,

In the office of the Librarian of Congress, at Washington.



## PREFACE TO THE SECOND EDITION.

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The speedy exhaustion of the first edition of these notes has led the author to issue another and enlarged edition. The nature of the work is expressed in its title. It has no claim to completeness, but is given as a guide to the student, who may desire to pursue this branch of study. In the preparation of these notes, various writers have been consulted; but especially is the author indebted to the following: M. Foster, T. Lauder Brunton, J. Burdon-Sanderson, F. W. Pavy, K. B. Hoffmann, Hoppe-Seyler, and Gornp-Besanez. The various writings of these distinguished physiologists have been the basis of the author's study for years, and whatever of merit these pages may contain is in great part due to the above-mentioned names.

The plates representing the crystals of the most important substances discussed in these notes will soon be issued on charts with references to the pages of this book under each cut. This has been decided to be better than placing the cuts in the text, for the following reasons: (1) the chart will be more convenient for constant reference, as it can be framed and preserved indefinitely; (2) it is intended to issue a series of these charts illustrating the majority of the crystals met with in the study of chemical physiology and pathology.

These notes have been especially prepared for the use of the students of the author, and embrace the work done by them.

ANN ARBOR, MICHIGAN, January, 1879.

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## PREFACE TO THE THIRD EDITION.

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So short a time has elapsed since the appearance of the second edition of this little book that many changes have not been necessary. The title has been somewhat modified to suit the changes made. A number of new subjects have been introduced, and at the request of many using the work the cuts have been bound with the text. The author has freely consulted standard works and journals upon the various subjects discussed. Thanks are due the editors of medical and scientific journals for the words of encouragement and suggestions of value, which the author has gladly received. Especial thanks are due Prof. J. B. Marvin, of Louisville, who has kindly gone over the entire text and given the author valuable aid.

ANN ARBOR, MICHIGAN, September, 1880.

# CHEMICAL PHYSIOLOGY AND PATHOLOGY.

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## DIGESTION.

§ 1. All living things absorb and excrete matter. Thus, the plant takes up carbonic acid and gives off oxygen. That microscopic speck of albuminous matter, known as the moneron, thrusts out any part of its body and takes in its food, digests it, utilizes a part and casts off the remainder. Many of the lower forms of living beings absorb their food directly from the surrounding world and without first subjecting it to any particular changes. The plant absorbs gases from the atmosphere and thus builds up its tissues. The gases, previous to absorption, are not subjected to the action of any digestive fluids secreted by the plant. However, there is a digestive process going on in many plants whereby substances otherwise insoluble are dissolved by the juices of the roots and thus fitted for absorption. Moreover, as Charles Darwin and others have shown, there are several species of plants, which digest animal substances by means of a secretion whose active principles are identical with, or cannot be distinguished from, those of the gastric juice of the higher animals. Man resembles the plant inasmuch as important articles of nutrition are received directly from the inorganic world. Oxygen, inhaled by animals, enters the circulation, and takes part in the various changes which support life. Oxygen is a food, but it is absorbed without the action of digestive fluids.

The process of digestion consists in certain physical and chemical changes which food undergoes while in the alimentary canal and whereby the nutritive parts of the food are fitted for absorption. The foods of man are inorganic, starchy, fatty

and albuminous. So far as digestion is concerned, we need not bestow much consideration upon the inorganic foods, since some of these are absorbed unchanged and the changes which take place in others are simple and in no way to be distinguished from those produced by various physical and chemical agents outside of the animal world. The digestive fluids, to which these foods are subjected, are the saliva, the gastric juice, the pancreatic juice, the bile and the intestinal juice. The different foods are affected variously by the several juices.

§ 2. *The Saliva.*—(a) In the mouth, food is masticated and mixed with the saliva. The mixed saliva is furnished from four sources, the parotid, submaxillary and sublingual glands, and the mucous membrane of the mouth. The saliva from these sources varies in its composition and the intensity of its action upon food. The parotid saliva of man is a clear fluid, of specific gravity from 1004 to 1007. It contains no morphological elements, but upon standing deposits calcium carbonate, which in the recently-obtained secretion is held in solution by carbonic acid gas. Parotid saliva contains from one to one and a-half per cent. of solids. Of these, about one-half are inorganic constituents, the most interesting of which is potassium sulphocyanate; besides this, there are traces of alkaline chlorides, phosphates and sulphates and calcium bicarbonate. The most important of the organic constituents is ptyalin: while an albuminous substance coagulable by heat is present. Parotid saliva may be obtained by inserting a canula into Steno's duct. On account of the large amount of carbonic acid gas it contains, the first few drops thus obtained will generally be found acid in reaction; but as the flow becomes more profuse, the reaction becomes feebly alkaline.

(b) The secretion of the submaxillary gland varies with the means by which the gland is excited; thus, by excitation of the chorda tympani or by irritating the tongue with a drop of acid, a peculiar secretion known as *chordal* saliva is obtained. On the other hand, by irritation of that branch of the cervical sympathetic which supplies this gland or by irritation of the tongue with pepper or an alkali, a different secretion, known

*sympathetic* saliva, appears. Again, if all the nerves supplying this gland be severed, or if their function be destroyed by curare, a saliva differing in composition from either of the others and known as *paralytic* saliva is secreted.

Chordal saliva is a clear, strongly alkaline fluid, with a specific gravity varying from 1003 to 1005. It contains globulin and traces of alkaline chlorides and phosphates and calcium bicarbonate. In the cat, the chordal saliva is more viscid than the sympathetic.\* Sympathetic saliva is cloudy with morphological elements and its specific gravity is about 1008. Paralytic saliva is poorer in solids than either of the other two. It has an alkaline reaction and specific gravity from 1001 to 1002.

(c) Saliva from the sublingual gland is tenacious and ropy, alkaline in reaction and contains ten per cent. of solids. Mucus is present in considerable quantity and to this constituent the viscosity of this secretion is due. Calcium bicarbonate is present in small quantity, but is not deposited in a crystalline form as it is in parotid saliva. The secretion of the mucous membrane of the mouth resembles sublingual saliva, since both are rich in mucin. The former contains epithelial scales, salivary corpuscles, and at times traces of cholesterin. Fat may also be present either from the food or from a diseased condition of the mucous membrane.

(d) Upon all solid foods, saliva exerts a physical influence, rendering the formation of a bolus possible and deglutition more easy. Upon the starchy food only, does saliva exert any marked chemical action. Under the influence of the peculiar ferment, ptyalin, starch takes up water and is converted into dextrin and sugar. This sugar is generally considered as identical with glucose or grape sugar; but it is less powerful in the reduction of copper and more powerful in the rotation of polarized light. In consideration of the manner of its formation, Seegen† has adopted the name, *ferment sugar*. It resembles maltose very closely.

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\*Langley, *Journ. Physiol.*, 1, 96.

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†Pflüger's *Archiv.*, B., XIX.

Starch consists of cellulose and granulose. The former is not colored blue by the action of iodine alone; but it is so colored by iodine after being subjected to the action of sulphuric acid. Granulose is colored blue immediately by the application of iodine. In the starch grain, cellulose and granulose are arranged in alternating layers. The saliva acts upon the granulose, but is without action on the cellulose. Consequently, raw starch is acted upon very slowly by saliva, since the coats of cellulose must be penetrated; but if the grains be ruptured by boiling, the granulose is exposed and is rapidly converted by the saliva into dextrin and sugar. Thus, during mastication a part of our food is converted into sugar, or is fitted for absorption. It must be understood that the short sojourn of the food in the mouth is not sufficient for the conversion of *all* the starch.

The inorganic constituents of the food, which are soluble in slightly alkaline fluids, are dissolved in the saliva. The fats are slightly emulsified and the proteids are not chemically affected.

§ 3. *Secretions of the Stomach.*—During its passage through the œsophagus, no part of the food is materially changed. The stomach furnishes two secretions which differ essentially in their composition and action upon foods. These are known as the *succus gastricus* and the *succus pyloricus*. As its name implies, the latter is secreted from the pyloric extremity of the stomach; while the *succus gastricus* is poured from the walls of the fundus of the same organ. The flow of the pyloric secretion is constant; while that of the true gastric juice is intermittent.

The *succus pyloricus* is a viscid, yellowish fluid, of alkaline reaction, specific gravity about 1010, and contains from fifteen to twenty per cent. of solids. When pure, it is without action upon albuminous food. However, after it has been rendered acid with dilute hydrochloric acid, this juice digests albumen with readiness. It has no action upon fats. The statements in regard to its amylolytic action are contradictory, and the subject needs further investigation.



The gastric juice is colorless, has a specific gravity which varies from 1001 to 1002, and does not contain more than one per cent. of solids. It is poured out during digestion, and has an acid reaction which is soon imparted to the entire contents of the stomach. Besides the free acid, which normally is hydrochloric, a ferment, pepsin, is present. By the combined action of the acid and pepsin, assisted by the movements of the stomach, the albuminous parts of the food are changed. The principal products of stomachic digestion are *peptones* and *parapeptones*. Various kinds of peptones have been described by authors; but for our purpose, it is, at present, necessary to note only the broad distinctions. The great physiological difference between peptones and parapeptones, is that the former are ready for absorption, while the latter must be farther changed before they can enter the circulatory system. Besides pepsin the gastric juice contains another ferment, rennet, which digests the casein of milk.

Upon starch the gastric juice has no effect, and often the acidity is so great as to arrest the action of the saliva upon this part of our food. Fat itself is not chemically changed by the gastric juice. When fatty food is taken, the albuminous envelopes of the globules of fat are digested in the stomach, and the fat thus freed from its proteid covering, is the more readily acted upon by the juices with which it meets in the intestines. From this fact, a practical lesson in physiology may be learned. It is very necessary to healthy digestion that the proteid envelopes of the fat should be digested in the stomach. In order that this may be fully accomplished, the fat of the food should be well distributed. If lumps of fat be swallowed, the gastric juice does not gain access to all the proteid matter, and consequently the fat still enveloped with albuminous matter passes into the intestines. It is true that the pancreatic juice acts upon proteids, but this action is slow unless the proteid has been previously converted into a parapeptone by the action of the gastric juice. If a lump of butter be swallowed, a disagreeable sensation and probably nausea will be produced; while if the same amount of butter be spread upon bread, the whole may

be eaten and relished. Children, who refuse fat meat will frequently consume a quantity of butter, containing several times as much fat as the meat refused. Fats constitute a very important and necessary part of our food, and if prepared properly never interfere with healthy digestion.

Some albuminous articles of food are digested more readily than others. Generally the rapidity with which proteids are digested in the stomach is in direct proportion to the comparative extent of (service) exposed directly to the action of the juice. Muscular fibre is dissolved much more rapidly than an equal weight of hard-boiled egg; because, the first readily separates into parts and is permeated by the juice, while the second is acted upon only from the outside. Again, unboiled albumen forms a clot, when taken into the stomach, and is dissolved with more difficulty than albumen which has been coagulated by heat. But if the raw white of the egg be shaken well with air, the bubbles of gas prevent the formation of a dense clot, and thus render the albumen more susceptible to the action of the digestive fluid.

The question is frequently asked, if the gastric juice dissolves albuminous food, why are not the walls of the stomach digested by their own secretion? This does occur, sometimes, after death. It has already been stated that the acidity of the gastric juice is normally due to hydrochloric acid. This acid is supposed to be obtained from the sodium chloride of our food. Under the influence of the peptic glands a chemical reaction between sodium chloride and water takes place, whereby free hydrochloric acid and sodium hydrate are formed. The sodium hydrate permeates the walls of the stomach, prevents their digestion by imparting an alkaline reaction, is taken into the blood and unites with carbonic acid, forming a carbonate. This carbonate is supposed to be carried to the liver and there to enter into new combinations, whereby the base for sodium glycocholate and taurocholate is furnished. This theory, which is especially insisted upon by Thudichum, may account for one of the causes which prevent the digestion of the walls of the stomach; but there are other and equally important

conditions which must be considered. In the first place, the fact that the walls of the stomach are permeated by blood-vessels containing an alkaline fluid must be recognized as a prevention of digestion of the organ itself. In the second place, it has already been stated that the secretion of the pyloric extremity of the stomach is alkaline. Moreover, this secretion is constantly being poured out, while the production of gastric juice is not continuous. Consequently, for the greater part of the twenty-four hours, the reaction of the mucous membrane of the stomach is neutral or alkaline. It is true that several observers have reported that the mucus of the dog's stomach examined through a fistula is constantly acid, even after a fast of many days' duration. Others have found the mucus neutral or alkaline in healthy dogs during the intervals of digestion. My experience belongs to the latter class, and I have never obtained an acid reaction in the empty stomach, after the wound made for the establishment of the fistula had completely healed. However, in the few cases, where an accidental fistula in man has afforded an opportunity of investigation, the mucus of the stomach has invariably been found neutral or alkaline when digestion was not going on. Again, the greater specific gravity of the pyloric secretion, together with its viscosity, may aid in protecting the walls. Indeed it has been ascertained by testing the reaction at different depths in the long glands of birds, that the acidity is confined to the surface.

The food which has been fitted for absorption by the action of the saliva and the gastric juice, is, in part at least, absorbed directly from the stomach. The chyme, as it passes through the pylorus, is rich in parapeptones, but contains little or no peptones. The latter, together with glucose, has been taken up by the capillaries, and thence into the gastric veins.

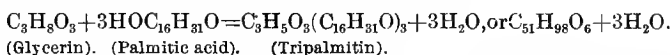
§ 4. *Pancreatic Juice*.—Almost immediately after leaving the stomach, the food is acted upon by the pancreatic juice. This digestive fluid produces changes in the starchy, albuminous and fatty food. Starch is quickly and completely changed into sugar by the action of the pancreatic juice. Thus, the process, began in the mouth, is completed in the

intestines and in a healthy condition, all the starchy food is now fitted for absorption. The para-peptones prepared in the stomach, are farther changed in the intestines into leucin, tyrosin, asparagic acid, glutamic acid and indol. Leucin is amidocaproic acid, has the formula,  $\text{NH}_2, \text{C}_5\text{H}_{10}, \text{CO}_2\text{H}$ , and is related to a true fat. Thus, we see that before the food has been taken into the system, the complex albumen yields leucin which is known to be a link in the chain of retrograde metamorphosis. Already, the chemical changes of the body have brought a part of the food from the condition of the highly complex albuminous molecule to that of the fatty: from its high position in the organic world, one step nearer the confines of inorganic nature. Tyrosin belongs to the group of aromatic bodies, bears a close relation to benzoic acid, and has the formula,  $\text{C}_9\text{H}_{11}\text{N O}_3$ . Glutamic acid has the composition represented by the formula,  $\text{N H}_2, \text{C}_3\text{H}_5 (\text{C O}_2\text{H})_2$ ; while asparagic acid is known as  $\text{N H}_2, \text{C}_2\text{H}_3 (\text{C O}_2\text{H})_2$ . The latter is easily obtained by boiling asparagus with alkalis. Both glutamic acid and asparagic acid may be prepared by digesting plant-fibrin with dilute acids. All of the albumen of the food is now fitted for absorption: the first change in the proteids, taking place in the stomach, where peptones and para-peptones were formed. The peptones were absorbed from the walls of the stomach: the para-peptones as such could not be absorbed and consequently passed into the intestines. Here by the action of the pancreatic juice, the para-peptones are farther changed, as has been stated above.

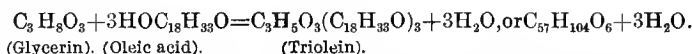
If albumen be directly digested with the pancreatic juice, peptones, leucin, tyrosin, glutamic acid, asparagic acid and indol are produced. The albumen is first broken up into peptones and para-peptones, and the latter is instantly decomposed, a change which could not have been produced by the action of gastric juice. However, there is a difference between the peptones formed by the gastric juice and those produced by the action of the pancreatic secretion. In the former instance, the peptones are acid albumins; while in the latter they are alkali-albumins. They are, however, physiologically

identical, since both are absorbable. Consequently, if a small amount of albuminous food escapes digestion in the stomach, it is acted upon by the pancreatic juice.

The pancreatic juice emulsifies fats and splits up neutral fats into glycerin and fatty acids. Thus, palmitin, or more properly tripalmitin, which exists as a fat in our food, consists of palmitic acid combined with glycerin. Glycerin is represented by the formula,  $C_3H_8O_3$ . When an acid combines with glycerin, the former replaces one or more of the atoms of hydrogen in the latter. There are three of these atoms replaceable and consequently the formation of a neutral compound requires the substitution of an acid for the three atoms of hydrogen. Palmitic acid is monobasic; therefore, three parts of this acid are required in order to combine with the glycerin, and thus form the neutral fat, tripalmitin:



Olein or triolein, another fat of our food and a constituent of olive and other oils, consists of oleic acid combined with glycerin:



Tristearin has a composition similar to the two fats mentioned above. Now, the pancreatic juice has the power of decomposing these neutral fats into their constituents, glycerin and fatty acids. The fatty acids, thus freed, combine in part with alkalis forming soaps and in this condition are absorbed. Other portions of the fatty acids are emulsified; the formation of the emulsion being hastened by the presence of sodium phosphate, which is contained in the food, or is furnished by the bile. If palmitic or stearic acid be boiled with sodium phosphate, a fine emulsion is formed; while if neutral fats be substituted for the fatty acids, the salt of sodium fails to produce any effect. Thudichum thinks that the chief effect that the bile has upon the absorption of fats, is due to the presence of sodium phosphate in the biliary secretion.

In some instances, the pancreas becomes diseased and fails to perform its function. When this is the case, much of the fat of the food is excreted unchanged with the fæces; nutrition is necessarily imperfect; the patient becomes very anæmic, and often, especially in the latter stage of the disease, is unable to retain any food. In one case of this kind I found that the pancreas had undergone fatty and calcareous degeneration. In this case, but little food could be retained for six weeks previous to death. Even a drink of ice water caused nausea and vomiting, and food was administered per rectum. The vomited matter had a peculiar coffee-ground appearance, which caused some physicians to diagnose cancer of the stomach, an error revealed by the postmortem examination.

§ 5. *Action of Bile.*—Bile, by virtue of its alkalinity, aids the pancreatic juice in the action of the latter upon food. As the food passes through the pylorus, the gall-bladder contracts and the bile rushes into the intestine. This is probably due to reflex action and the flow of bile can be produced by irritating the pyloric orifice with any acid solution, but not with alkalis. The bile assists in destroying the acidity of the food; precipitates the parapeptones and carries down mechanically with this precipitate any pepsin that may have passed from the stomach with the food. Bile furnishes bases which unite with the fatty acids forming soaps. Evidently, bile aids the absorption of fats; thus fats pass more readily through animal membranes, or filter papers which have been moistened with bile, than through membranes or papers moistened with water. Assisted by one of my former students, Mr. Worden, I made quite a number of experiments upon the effects of bile upon the absorption of fats from the intestines. Animals, cats and dogs, were put under the influence of chloroform, the walls of the abdomen were opened and loops of the intestines ligated, care being taken not to separate the intestine from its attachments. The first object was to find from what part of the small intestines, fats were absorbed with the most readiness. For this purpose, a section of two inches of intestines near the pyloric extremity of the stomach was ligated at each end, a

second and similar section in the lower part of the duodenum, and still a third, near the termination of the small intestines, were ligated in the same manner. In all cases, the section was freed from its contents by gentle pressure. Into each of these knuckles of intestine, were injected equal volumes of cod liver oil. After from four to six hours, each section was removed and the oil remaining in it was estimated. It invariably occurred that the section from the lower part of the duodenum contained less oil after removal, than either of the other sections.

In the second place, contiguous sections at the lower part of the duodenum were ligated as above. Into one of these, oil containing bile, and into the other oil freed from bile were injected. Upon removal of the sections, and estimation of the remaining oil as above, it was invariably found that the presence of bile had favored absorption of the oil. However, the proportion of bile to the oil, most suitable for absorption, seemed to be limited; thus the addition of an undue amount of bile to oil did not hasten the passage of the latter through the intestinal walls.

In some animals, at least, bile has the power of converting starch into sugar; but this action is so slight and the same property is possessed in a similar degree by so many animal fluids which are in no way concerned in digestion, that it may be considered as of little importance.

§ 6. *Intestinal Juice*.—The reports of experimenters upon this fluid are often contradictory, and it is quite probable that the juice differs in composition and physiological action in different animals. In case of an intestinal fistula in the human subject, Busch found that the intestinal juice digested proteids and converted starch into sugar, but had no effect upon cane sugar, nor did it emulsify fats. The juice obtained from the intestine of the pig and rabbit converts both starch and cane sugar into grape sugar; while, according to Bernard, the only ferment characteristic of the intestinal juice is *invertin* by virtue of which cane sugar is converted into invert sugar or *lævulose*.

That there are certain fermentative changes going on in the intestines and due to the presence of bacteria is certain.

## ANALYSIS OF SALIVA.

### MICROSCOPICAL EXAMINATION.

§ 7. Examine a drop of saliva under the microscope. Observe mucous corpuscles and pavement epithelium, and, if the mouth has not been kept clean, particles of food, cryptogamic sporules, and sometimes vibrios.

### METHODS OF OBTAINING MIXED SALIVA.

A quantity of saliva, sufficient for analysis, may be obtained by artificially stimulating the glands. This may be done in either of the following ways :

1. By attempting to chew a glass stopper.
2. By depressing the lower jaw and tickling the fauces with a feather.
3. Fill the mouth with vapor of ether, carry it back into the pharynx and retain it for some time.
4. Touch the end of the tongue with a crystal of citric or tartaric acid, or with one of sodium carbonate.
5. Exert a strong pressure under the chin, and at the same time tickle the palate with a feather.

*General Properties.*—The mixed saliva is turbid, bluish-white, and devoid of taste and odor. The normal reaction is alkali, and during mastication, the alkalinity is increased; but while fasting, it again gradually decreases until just before the next meal, when it may be neutral, or even faintly acid. In some cases of diseased saliva, especially when the flow is scanty, or when the person suffers from dyspepsia the reaction is constantly acid. The specific gravity varies from 1002 to 1009, the usual variation being between 1004 and 1006. Upon standing for some time, saliva forms a grayish-white deposit, which by examination with the microscope, will be found to consist of leucocytes and pavement epithelium. Owing to the mucin which it contains as a normal ingredient, saliva is somewhat viscid, and can be drawn out into threads after having been stirred briskly for a few moments with a glass rod. No other



animal fluid decomposes more readily than this, consequently it is necessary that all specimens for examination in physiological research, or for diagnostic purposes, should be perfectly fresh. A disregard of this fact caused Wright (Lancet, 1842,) to ascribe to saliva a sharp, saline, and slightly astringent taste, and the property of poisoning vegetable and animal organisms (Lehmann.) The amount of saliva secreted by a healthy adult varies from one thousand to two thousand grams for the twenty-four hours.

#### INORGANIC CONSTITUENTS.

§ 8. In order to obtain tests for the inorganic constituents the saliva must be filtered ; but as it decomposes so rapidly and filters so slowly, some caution is necessary. Everything used must be perfectly clean, for the precipitates will be so slight that they may not be seen ; or, what is more likely to occur, one may think that he has a sufficient test for some base or acid, when the turbidity is solely due to the test tube not being clean, or the filter paper containing some impurity. Before passing the saliva through the filter, it would be well to pass several ounces of water through and then test the water for each of the constituents soon to be given. Of course, in this case, all the results should be negative. Having tested the filter paper in this way, the saliva is mixed with about three times its bulk of boiling distilled water, and filtered. The filtrate may now be tested as follows :

*For Chlorides.*—Acidify a part of the filtrate strongly with nitric acid, and add a few drops of silver nitrate : the appearance of a white precipitate insoluble in acids, but soluble in ammonium hydrate indicates the presence of hydrochloric acid.

*For Sulphates.*—Acidify a part of the filtrate with hydrochloric acid, and add a few drops of barium chloride solution : insoluble barium sulphate will appear.

*For Phosphates.*—To some of the filtrate add a few drops of sodium acetate, and then some uranic acetate : a yellowish-white precipitate, insoluble in acetic, but soluble in hydro-

chloric acid, shows that phosphoric acid is present, and has been precipitated as uranium phosphate.

*For Calcium.*—Calcium will be precipitated as an oxalate upon the addition of ammonium oxalate.

*For Magnesium.*—This will appear as an ammonio-magnesium phosphate, upon adding to the clear filtrate some ammonium hydrate, ammonium chloride and disodium hydrogen phosphate.

*For Sulphocyanic Acid.*—This acid is not always present. It is derived from the parotid gland, and is not always found in the secretions of the other glands. It should be tested for by distilling 300 c. c. of saliva, rendered acid with dilute sulphuric acid; neutralizing the concentrated distillate with sodium hydrate and adding a drop of dilute ferric chloride, when, if sulphocyanic acid be present, a blood-red color will be produced.

This acid may also be obtained by the following method:

Evaporate the saliva to dryness on the water-bath, treat the residue with alcohol and filter, evaporate this filtrate, dissolve the residue thus obtained in a little water, and test this solution with ferric chloride, as in the preceding method.

The amount of sulphocyanic acid may be estimated by heating the aqueous solution of the alcoholic extract with potassium chlorate and hydrochloric acid, and precipitating the sulphuric acid, thus formed, from the sulphocyanic acid, with barium chloride, drying and weighing the precipitate.

In some cases the blood-red color may be obtained upon the addition of ferric chloride directly to the saliva. If the reaction fails when the test is applied in this way, it is no proof that sulphocyanogen is wholly absent; while on the other hand, if the red color is produced by the direct application of the ferric salt, it must be remembered that this alone is not positive proof of the presence of sulphocyanide; for the perchloride of iron produces the same color with meconic acid, which may be present in the patient's mouth from opium. Consequently a farther test is necessary, and any doubt may be removed by the addition to the colored solution of a little

mercuric chloride, when, if the color had been produced by a sulphocyanide, the solution will become colorless; while, if meconic acid be present, the mercuric chloride will cause no visible change. Ferric salts also strike a red color with strong acetic acid, with a decoction of mustard, and with an infusion of Iceland moss; but these are never present in quantities sufficient to give the reaction, and even if this were possible, they would be recognized by their other properties.

*For Nitrous Acid.*—Sometimes mixed saliva contains nitrous acid as nitrite. Add to the saliva a little cooked starch, some potassium iodide solution, and a few drops of sulphuric acid, stirring well; when, if nitrous acid be present, the mixture will be colored blue by the formation of starch iodide.

*For Sodium and Potassium.*—Evaporate a small dish full of saliva to dryness on the water-bath. Place some of the residue thus obtained on a platinum wire and heat it in the colorless flame of a Bunsen burner. The flame as seen through a blue glass, presents the violet color, characteristic of potassium; while without the glass the flame is seen to be of a yellow color, due to the presence of sodium. These bases are, in part, combined with the acids already referred to, and partly with organic substances. The latter combination is feeble, and the organic substances are freed directly upon the addition of any inorganic acid; as for instance, it will be seen under ptyalin that on the addition of phosphoric acid, this substance is set free, and falls with other precipitated matter.

*Determination of the Amount of Water and Solids.*—Place a small crucible with its cover in an air-bath or box water-bath and keep at 100° C. for half an hour. Remove the crucible to a dessicator, which contains a dish of sulphuric acid, and after the crucible has cooled, weigh it. Again put the crucible in the bath and keep it at 100° for another half hour, cool in the dessicator and weigh as before. This must be repeated if necessary, until the weight is constant. Then fill the crucible two-thirds full of saliva and weigh again. The difference between the weight of the crucible containing the saliva and that of the empty crucible, will be the weight of the saliva.

Place the crucible containing the saliva, after being weighed, in the bath, and keep at  $100^{\circ}$  until all the water has been driven off. Cool in the dessicator and weigh. Repeat the heating, cooling and weighing, until the weight remains constant. The difference between the weight of the crucible containing the saliva and that of the crucible with the residue will be the weight of water in the saliva taken. From this the per cent. of water must be calculated. The difference between the weight of the crucible with the residue and that of the empty crucible, will be the amount of solids in the saliva taken, and from this the per cent. of solids may be obtained.

Place the crucible with the residue over a Bunsen burner and keep at a red heat for half an hour, cool in the dessicator, weigh and repeat this operation until the crucible ceases to lose any weight. By the continued application of heat the organic constituents of the total residue have been driven off, and only the inorganic matter is left. The difference between the weight of the crucible with the total residue and that of the crucible with the inorganic residue is the weight of organic matter in the saliva taken; while by subtracting the weight of the empty crucible from that of the crucible with the inorganic residue, the weight of the latter is obtained. From these results the per cent. of organic and inorganic solids should be calculated.

#### ORGANIC CONSTITUENTS.

§ 9. The principal organic constituents are albumen, mucin, and salivary diastase or ptyalin.

*Albumen.*—If saliva be strongly acidified with nitric acid, it becomes more turbid. If it then be boiled, the coagulum takes a yellow color and is not dissipated, thus showing the presence of albumen. A confirmatory test may be obtained by adding to a second portion of saliva a mixture of acetic acid and potassium ferrocyanide, when a white precipitate is produced.

*Mucin.*—The tenacity of saliva is due to mucin. To some saliva in a small beaker add gradually acetic acid, stirring with a glass rod; the mucin separates in white stringy flakes.

*Ptyalin*.—Collect 600 c. c. of saliva, acidify it strongly with phosphoric acid, then add milk of lime till the mixture is faintly alkaline, and filter. The ptyalin is now on the filter paper, but contains many impurities. Remove the filter paper with its contents to a clean beaker, and add distilled water not exceeding in quantity the saliva originally employed; stir well and filter again. The ptyalin is now in the filtrate, and may be precipitated by the addition of absolute alcohol, and dried over sulphuric acid.

*Ptyalin from the Salivary Glands*.—As ptyalin exists already prepared in the salivary glands, it may be obtained from these more easily and in greater quantity than from the saliva. Cut the salivary glands of any animal into very small pieces, place these in a flask, and cover with absolute alcohol. Cork the mouth of the flask and let it stand for twenty-four hours. Pour off the alcohol and press the remainder in a cloth, in order to remove as much of the alcoholic extract as possible. The cake thus obtained is placed in a beaker, covered with glycerin, and allowed to remain for several days, being thoroughly stirred occasionally. It is then strained through a cloth and afterwards through paper. From this filtrate, ptyalin is precipitated by absolute alcohol.

*Amylolytic Action of Saliva*.—To some filtered saliva, add a few drops of Fehling's solution (or some dilute solution of sulphate of copper and then an excess of potassium or sodium hydrate.) A blue precipitate is thrown down and on being boiled, the solution takes a pale rose color from the action of the copper solution on the albumen, but the copper is not reduced. This shows that sugar and other substances which reduce copper are not present in normal saliva. Now boil one gram of starch in one liter of distilled water, and filter. To some of the filtrate in a test tube add some Fehling's solution. A blue precipitate falls, and on boiling the solution becomes black. Again the copper is not reduced. Now to some filtered saliva add twice as much of the starch solution and place the mixture on the water-bath and keep at about 40° C. for some minutes; then to some of this mixture add Fehling's solu-

tion and boil. A yellow or yellowish-red precipitate of the suboxide of copper appears. The starch has been converted, by the action of the saliva into sugar, which reduces the copper. If the saliva, before being mixed with the starch, is heated to  $60^{\circ}$  or  $70^{\circ}$ , its power of converting starch into sugar is lessened, and if it be boiled this power is wholly lost. The amylolytic action of saliva is arrested by freezing or by the addition of strong acid, but is regained by raising the temperature and by neutralizing the acid. Caustic potash and soda destroy the action of saliva on starch, and in this case it is not renewed by neutralization. The carbonates of these alkalies arrest the diastatic power, which is restored upon carefully adding an acid until the neutral point is reached. At ordinary temperature starch is converted into sugar by saliva, but the change goes on most rapidly at about  $40^{\circ}$  C. The rapidity of the conversion is also influenced by the kind of starch. Instead of saliva, a solution of ptyalin, prepared according to either of the methods already given, may be used in all the cases referred to.

*Nature of the Change.*—The chemical change taking place in the starch during its transformation into sugar is due to a process of hydration. The starch takes up water and is converted into sugar and dextrin (the erythrodextrin of Brücke.) If at this stage, a solution of iodine in potassium iodide be added to the mixture, a red or violet coloration, (hidden more or less by blue if unchanged starch be present), due to the action of the iodine on the dextrin, will be produced. By the farther action of the saliva, the erythrodextrin is split up into sugar and another dextrin (the achroodextrin of Brücke.) Achroodextrin is not colored by iodine and is not changed into sugar by the action of the saliva. It may be that the starch is first split up into sugar and the two kinds of dextrin, the erythrodextrin being afterwards converted into sugar, while the achroodextrin is not changed. However this may be, the final products of the action of saliva upon starch are sugar and dextrin.

#### ABNORMAL SALIVA.

§ 10. *Iodine and Bromine.*—The saliva may be filtered

as directed and the filtrate tested for iodine and bromine, with chlorine and carbon disulphide. I have detected iodine in the saliva within five minutes after the administration of a ten grain dose of potassium iodide. Besides iodides and bromides, many other medicinal substances appear in the saliva.

*Mercury*.—Slightly acidify the filtrate, and place in it a strip of clean copper; metallic mercury will be deposited upon the copper. During salivation from mercury, sulphocyanogen disappears from the saliva.

*Urea*.—Evaporate the filtrate almost to dryness, then add nitric acid equal in bulk to the part left. Set aside in a cool place for five minutes and at the expiration of this time examine under the microscope. Flat rhomboidal crystals of urea nitrate will appear. Urea appears in the saliva only after the kidneys have ceased to perform their duties.

*Urates*.—Evaporate some of the filtrate to one-half its bulk, then acidify with nitric acid, only using enough to make strongly acid, and after five minutes examine under the microscope for crystals of uric acid; also apply murexide test. (See under Urates in Urine.)

*Pus*.—May be detected under the microscope, the corpuscles are identical with the white corpuscles of blood, and upon the addition of acetic acid, present from one to three, generally three, nuclei. The albumen will also be increased in saliva containing pus.

*Excess of Chlorides*.—An excess of chlorides will be indicated by the bulk of the precipitate obtained with silver nitrate.

*Excess of Phosphates*.—Will be shown by bulk of precipitate with uranic acetate. The phosphates are often deposited upon the teeth.

*Excess of Carbonates*.—Shown by brisk effervescence with acetic acid. The carbonates, when the saliva is being poured into the mouth, are dissolved in an excess of carbonic acid, which escapes, and the carbonates are then deposited upon the teeth, forming tartar.

*Bile*.—Saliva containing much bile, takes a dull, yellowish

color, gradually deepening into a faint olive color, upon the addition of nitric or hydrochloric acid. The tests for bile-acids and bile-pigments should also be made. (See under Bile.) Dr. Fenwick (London Lancet, September 1, 1877,) calls attention to the examination of the saliva for bile in "billiousness." The patient complains of his liver being out of order, and of a bitter taste in his mouth on rising in the morning. His skin and conjunctivæ are not yellow, and the examination of the urine fails to reveal the presence of bile. But if an ounce of the saliva be evaporated to dryness on the water-bath, a yellow or reddish-brown residue is left, which is soluble in chloroform and gives the reaction of bile-pigment. Upon examination, the back part of the patient's tongue on rising in the morning will also be found colored yellow or reddish-brown. During the night the heat of the mouth evaporates the saliva (which in waking hours is swallowed) and affords the same indication of bile as is obtained by evaporation on the bath. A smaller quantity of bile can be detected in the saliva than in the urine, because the normal coloring matter of the latter interferes with the test. The physician who fails to make use of this means of diagnosis will often fail to recognize the hepatic derangement. In some cases of "billiousness," bile acid can be detected by evaporating two ounces of the saliva to dryness on the water-bath, treating the residue with boiling absolute alcohol, filtering, evaporating the filtrate to dryness, redissolving in water and applying to this solution Pettenkoff's test. But in the majority of instances, the quantity of bile-acids is too small to afford the test, and the physician must rely upon the colored residue and the bitter taste, which are sufficient proof of the presence of the hepatic secretion in the blood.

*Blood.*—The presence of blood in the saliva is shown by the color, which varies from red to black; also by the appearance of corpuscles under the microscope. It must be observed whether the blood comes from the cavity of the mouth or whether it is poured out with the saliva. This can be ascertained by carefully inspecting the parts.



*Tyrosin and Leucin.*—Concentrate the saliva without filtering, and examine under the microscope. Tyrosin will be found in needle-shaped crystals, which are not soluble in acetic acid, and in this way are to be distinguished from calcium phosphate. Leucin appears in globules resembling oil in appearance, but insoluble in ether. Tyrosin and leucin have been found in the saliva of hysterical persons.

*Milky Saliva.*—Opaque, curdy, and acetic acid increases the coagula.

*Sugar.*—Ferments with yeast, alcohol and carbonic acid being formed. Also reduces the copper solution, as given under urine.

*Excess of Fat.*—Evaporate some saliva to dryness on the water-bath, extract with ether and judge of the quantity of fat under the microscope.

*Acid Saliva.*—Indicated by action on litmus. Acid saliva contains lactic acid. Wright holds that acidity of the saliva may accompany any of four classes of diseases: (1) idiopathic affections of the salivary glands; (2) those diseases in which there is an excess of acid in the system, as rheumatism, scrofula, phthisis, rachitis and amenorrhœa; (3) inflammatory affections of the mucous membrane of the stomach and intestines; (4) in dyspepsia. Lehmann found the saliva always acid in cancer of the stomach and in diabetes mellitus, and frequently but not invariably, acid in catarrh of the gastric and intestinal mucous membranes and in ulceration of the stomach. Frerichs states that the acid reaction is always due to the secretion of the buccal mucous membrane.

§ 11. *Salivary Calculi.*—These are usually composed of calcium phosphate and carbonate and organic matter. I once analysed one which was two inches long and about one-quarter of an inch in diameter and weighed forty-eight grains. Upon boiling some of it with acetic acid, carbonic acid gas was given off and the disagreeable odor of putrid saliva was observed. On making a cross section of the calculus its interior was seen to be composed of layers of a chalky white substance, principally calcium carbonate. The surface was rough, and covered

with a greenish deposit of organic matter. Dental calculi form in decayed teeth of men and animals. They resemble salivary calculi in composition; but contain more organic matter and calcium phosphate. Dental calculi are often covered with, and form nests for vibrios. Tarter which forms upon the teeth is of the same composition as salivary calculi are.

### GASTRIC JUICE.

§ 12. For physiological purposes gastric juice is obtained artificially or by the establishment of fistulæ in the lower animals. For diagnostic purposes the physician must examine this secretion as contained in vomited matters. The normal gastric juice of man is a clear watery fluid, and contains but a small amount of solids. Its specific gravity varies from 1001 to 1010, and it contains about .2 per cent. of free hydrochloric acid. Heidenhain\* found the secretion of the isolated fundus of the dog's stomach to contain only 0.45 per cent. of solids, and as much as .5 per cent. of free acid. In some herbivorous animals the gastric juice has a brownish color and contains less acid and less pepsin than that of the carnivora. Normally the acidity of this secretion is due to hydrochloric acid and acid phosphates, but in certain diseased states these may be entirely replaced by lactic, butyric and acetic acids, one or all.

The action of gastric juice upon albuminous food is due to the combined effects of hydrochloric acid and pepsin. The latter has never been obtained in a perfectly pure state, consequently its chemical formula is not known.

§ 13. *The Free Acid.*—The following are some of the simplest and most satisfactory tests for the free acid of the gastric juice:

1. The color of a dilute solution of methylanilin violet is not altered by the addition of an organic acid, but by free mineral acid (markedly by HCl) is changed first to a blue, then green, and finally is decolorized. The application of this test will show that the gastric juice contains a mineral acid. (Maly).

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\*Pflüger's Archiv., B. xix, s. 148.

2. Amylic Alcohol does not dissolve mineral salts, but does dissolve the quinia salts of the mineral acids. Digest recently precipitated quinia for some hours with filtered gastric juice, at from  $40^{\circ}$  to  $50^{\circ}$ ; then evaporate to dryness and extract the residue with amylic alcohol; the residue from this extract contains crystals of quinia chloride which may be recognized by microscopical examination, or the crystals may be dissolved in water and the amount of hydrochloric acid determined with a standard solution of silver nitrate. Rabuteau found by this method 2.5 parts per thousand of free HCl in the gastric juice. (Rabuteau).

3. A solution containing starch, potassium iodide and potassium iodate is colored blue by hydrochloric, but is not affected by lactic acid. Such a solution is colored blue on the addition of gastric juice. (Rabuteau).

4. A very dilute solution of ferric acetate wholly free from alkaline acetate will remain unchanged in color on the addition of a few drops of a solution of potassium sulphocyanate; but on the further addition of a trace of mineral acid, the blood-red color of ferric sulphocyanate appears. HCl is very marked in its action. By this test the presence of free mineral acid in the gastric juice may be recognized. (Szabo).

5. Poison an animal with  $\text{Hg Cy}_2$ . Remove the stomach and subject its contents to distillation. The distillate will be found to contain HCl. Lactic acid or other organic acid would not have been sufficiently strong to decompose the  $\text{Hg Cy}_2$ , therefore the stomach contained a mineral acid. (Bellini.)

6. If an aqueous solution of an organic acid be shaken with ether, the acid will pass into the ether solution; while mineral acids are not removed from aqueous solution by ether. In one part of filtered gastric juice estimate the amount of free acid with a standard alkaline solution. Shake a second part of the juice with ether, and remove the ethereal layer. Now, with the alkaline solution estimate the amount of free acid in the juice which has been agitated with ether. It will be found that the ether has not removed any of the acid, which must therefore be a mineral acid. This is true only when the gastric

juice is fresh; for if it stand some time, and especially if it contain partly digested food, lactic acid will be present. (Richet).

§ 14. *Nature of the Acids.*—Certain cases of acute anemia are due to the abnormal condition of the gastric secretion. The same is true of gastric catarrh and certain febrile affections. The vomit of persons affected with these diseases is intensely acid, but contains neither hydrochloric acid nor pepsin. The nature of the acidity can be ascertained as follows: The vomited matter, if not sufficiently liquid, should be mixed with a little water and filtered. If the filtrate is not clear, filter again either through cloth or paper, or through both. Put the filtrate into a large retort, connected with a Liebig's condenser, and distil at about 130°. If during the process of distillation a thick scum should form over the contents of the retort, the liquid should be removed, freed from the pellicle by filtration, then replaced in the retort. Continue the distillation until the retort fills with a dense white cloud. Neutralize the distillate with sodium carbonate, evaporate it to dryness on the water-bath, extract the residue with absolute alcohol, filter the alcoholic solution, again evaporate to dryness on the water-bath, and dissolve this residue in a little distilled water. To a small portion of this aqueous extract, in a test tube, add a few drops of a neutral solution of ferric chloride; a blood-red color which is destroyed by the subsequent addition of hydrochloric acid appears if acetic acid be present. To a second portion of the watery extract, add silver nitrate; a white precipitate, insoluble in nitric acid, soluble in ammonium hydrate, shows that free hydrochloric acid was present in the substance under examination. To the remaining portion of the aqueous extract, add a few drops of dilute sulphuric acid, allow to stand for an hour, observing the odor from time to time. If butyric acid be present, the peculiar odor of rancid butter will be recognized.

Any lactic acid that may have been present in the matter under examination, remains in the retort. In order to ascertain the presence of this acid, shake the residue in the retort with

much ether, remove the ethereal layer with a pipette and evaporate this to dryness on the water-bath. Dissolve the residue in water and boil this solution with the oxide or carbonate of zinc. Remove the excess of zinc by filtration, concentrate the filtrate on the water-bath and allow to stand, when, if lactic acid were originally present, crystals of zinc lactate form in square prisms with one or two oblique surfaces at the ends.

Lactic and butyric acids are sometimes found in large quantities, as much as five grams of the two having been obtained. The latter is supposed to originate from the former by the liberation of  $\text{CO}_2$  and  $\text{H}$ . It very seldom or never happens that acetic, lactic, and butyric acids are all present. The process given above for detecting these acids in vomited matter, will also apply to the examination of gastric juice obtained through a fistula or the contents of the stomach after death.

§ 15. *Estimation of the Amount of Free Hydrochloric Acid.*—The amount of free hydrochloric acid present in gastric juice, the contents of the stomach, or vomited matters, is best ascertained by the method proposed by Schmidt, and which is as follows: To a measured amount of the clear filtrate (from which insoluble substances and albumen, if present, have been removed by heat and filtration) add nitric acid and silver nitrate. Collect the precipitated silver chloride upon a filter and wash well with distilled water. (Reserve the united filtrate and wash-water for further examination.) Dry the precipitate on the filter in an air or steam oven. Shake the dry silver chloride from the filter upon a piece of glazed paper. Burn the filter paper, allowing the ashes to fall into a small crucible, the weight of which has been previously ascertained. The silver chloride which adhered to the filter now exists with the ash and as metallic silver. To the ash add a few drops of nitric acid; this dissolves the silver forming the nitrate. To this add a few drops of hydrochloric acid which again forms the chloride. Evaporate the contents of the crucible to dryness at the temperature of the water-bath. To this residue add

the silver chloride which has been placed on the glazed paper. Again dry the crucible with its contents and weigh. From the weight of the whole subtract the weight of the crucible and the remainder will represent the weight of silver chloride. Every part of silver chloride will represent .247 parts of chlorine; from this the total amount of chlorine in the gastric juice or vomited matters taken, is calculated.

The filtrate from which the chlorine has been removed with silver nitrate, is now freed from any excess of silver by the careful addition of hydrochloric acid and filtration, then placed in a large crucible, evaporated to dryness, and the residue heated until all the organic matter is driven off. The ash is dissolved in water slightly acidified with acetic acid, and this solution, which may be diluted to any desired extent, is carefully measured and divided into five parts. It is not necessary that these five be equal parts, but the exact amount of each, and its relation to the whole must be noted.

In one of these parts the phosphoric acid is estimated volumetrically with uranic acetate as given under the quantitative examination of the urine.

In a second portion the sulphuric acid is estimated as follows: Render the solution strongly acid with hydrochloric acid and then add barium chloride as long as a precipitate is formed. Collect the precipitated barium sulphate on a filter, wash with hot water, dry, transfer to a weighed crucible, burn the filter paper, adding the ash to the contents of the crucible, heat to a dull red heat, cool over sulphuric acid and weigh; one part of  $\text{BaSO}_4$  represents .412 parts of  $\text{SO}_4$ .

In a third portion estimate the amount of calcium and magnesium as follows: Render the solution strongly alkaline with ammonium hydrate, then add ammonium oxalate. Heat the mixture gently and collect the precipitate upon a small filter. (Reserving the filtrate for the estimation of magnesium.) Dissolve the calcium oxalate on the filter in dilute hydrochloric acid. To this solution, concentrated if necessary to a small volume, add an excess of alcohol and then dilute sulphuric acid as long as a precipitate is formed. Collect the

precipitated calcium sulphate, wash with dilute alcohol, dry, transfer to a weighed crucible, burn the filter paper, heat the whole to redness, cool over sulphuric acid and weigh. Each part of  $\text{CaSO}_4$  represents .294 parts of calcium.

Concentrate the reserved filtrate, from which the calcium oxalate has been removed, to a small volume. Add ammonium chloride, ammonium hydrate and sodium phosphate. Cover the beaker containing the mixture with a piece of glass and allow to stand for twenty-four hours. Collect the precipitate upon a small filter, wash with a mixture of one volume of ammonium hydrate and three volumes of water. Dry the filter with its contents and transfer to a weighed crucible, burning the filter paper and adding the ash to the contents of the crucible. Heat the crucible to an intense redness. Dry over sulphuric acid and weigh. The magnesium was precipitated as ammonium-magnesium phosphate, which by the high heat has been converted into magnesium pyrophosphate, and in this form it is weighed. Each part of the pyrophosphate,  $\text{Mg}_2\text{P}_2\text{O}_7$ , contains .216 parts of magnesium.

In a fourth portion of the aqueous solution of the ash, estimate the amount of potassium and sodium as follows: Treat the solution with calcium chloride as long as a precipitate is produced, then add barium hydrate until the mixture has a feebly alkaline reaction. Remove the precipitated matters by filtration; to the filtrate add ammonium hydrate and carbonate as long as a precipitate forms. Remove the excess of calcium and barium now precipitated as carbonates, by filtration; wash the precipitate; evaporate the united filtrate and wash-water to dryness. Heat the residue to bright redness, and maintain this temperature for some time in order to drive off the excess of ammonium carbonate; cool; dissolve the residue in water; filter; wash any residue, that may rest on the filter, well with water. Unite the filtrate and wash-water; concentrate, if necessary; pour into a small weighed crucible; evaporate to dryness; heat the residue; cool and weigh. This gives the combined weight of the chlorides of sodium and potassium. In order to separate these bases dis-

solve the weighed residue in a little water, add some dilute alcohol and then platinum chloride as long as a precipitate forms. Cover and allow to stand for twenty-four hours. Then collect the precipitate on a small filter, dry at the temperature of the water-bath, and weigh. Each part of the double chloride of potassium and platinum,  $K_2PtCl_6$ , contains .306 parts of potassium chloride, KCl. The weight of the latter subtracted from the weight of the combined chlorides already found, gives the amount of sodium chloride. From the weights of their respective chlorides the amount of each base is calculated.

The fifth portion of the solution of the ash which has been held as a reserve in case any accident should happen during the examination of one or more of the other portions, is now, if the above estimations have been satisfactory, discarded.

The amount of each base and acid contained in certain measured portions of gastric juice, or extract of the contents of the stomach or vomited matters, is now known. From these figures the amount of each base and acid in the *same* quantity (100 c. c.) of the fluid is calculated. From the equivalence of each acid and base, the amount of the various salts are calculated, observing the following rules: 1. The sulphuric acid is to be considered as combined with potassium, forming  $K_2SO_4$ ; and any excess of this acid over the base is supposed to combine with sodium forming  $Na_2SO_4$ . 2. The phosphoric acid is to be regarded as forming acid phosphates,  $RH_2PO_4$ . In this formula, R represents calcium, magnesium, or sodium, one or all, in the order given. 3. Any remaining bases which may not have been taken up by the sulphuric and phosphoric acids are supposed to have existed as chlorides. 4. Any excess of hydrochloric acid remaining, existed originally as free acid.

The above method obviates the possibility of hydrochloric acid being set free by the action of lactic acid upon the chlorides of calcium and magnesium, this having been urged by some as the source of the free acid; and which may possibly happen if the old method of obtaining the acid by distillation be employed.

*Amount of Free Acid.*—The total amount of free acid may



be ascertained by means of a standard solution of sodium hydrate (as directed for the estimation of free acid in the urine).

§ 16. *Artificial Gastric Juice*.—1. Take the stomach of a recently killed animal, dog, pig, or fourth stomach of a calf; open and spread upon a board with the mucous side upward, Wash this with a gentle stream of water; then scrape off all the mucus; rub this up in a mortar with powdered glass and water; allow to stand for two hours; filter and dilute the filtrate with an equal bulk of a 0.2 per cent. solution of HCl. This will digest fibrin, and may be kept in closed bottles for a long time. 2. Remove the mucous membrane from the stomach of a pig, wash with water and cut into fine pieces. Cover these with dilute hydrochloric acid (made by adding 4 c. c. of hydrochloric acid to one liter of water); allow to stand for four hours, stirring the mixture frequently; filter off the fluid and extract the residue with another portion of the dilute acid. Repeat this process as long as the filtrate acts upon fibrin, as given below. In this way from one to six liters of an active extract may be obtained from the stomach of one pig. The juice thus obtained acts very energetically but contains such quantities of peptones that it decomposes in a few days.

§ 17. *Action of Gastric Juice*.—This is best shown upon fibrin from blood. Stir some fresh blood with a rough stick, a bundle of glass rods, or a piece of whalebone; collect the fibrin which has been coagulated; wash it until it is perfectly white; boil it in water and then collect again. Put a small piece of this fibrin into a test tube with some gastric juice, made as above, or obtained through a fistula, or by means of a stomach pump or gastric syphon, and keep on the water-bath at 35° to 40°C. The fibrin will swell and soon dissolve.

If small bits of coagulated albumen be substituted for the fibrin, the same results will be obtained. The rapidity of solution will depend upon the relative extent of surface exposed to the action of the juice.

§ 18. *Extraction of Pepsin*.—Cut open a stomach and wash as above, remove the pyloric part, and dissect off the remainder

of the mucous membrane. Cut this into small pieces, put into a beaker and cover with glycerin, allow to stand for two days; then strain off the glycerin. This will be found to have taken up the pepsin and gastric juice may be formed by adding to a little of the glycerin a 0.2 per cent. solution of HCl. That pepsin alone will not digest fibrin may be proven by diluting some of the glycerin extract with water and adding a piece of fibrin and keeping on the water-bath at 35°C.; the fibrin will not be dissolved. That the dilute HCl will not by itself digest the fibrin should also be proven. But as soon as the pepsin and dilute acid are mixed and the experiment tried, it is at once successful. These three experiments should be made at the same time, in as many test tubes.

The glycerin extract of the fresh pylorus contains no pepsin, as is evidenced by its failure to digest fibrin even after being properly acidified; but an infusion of the pylorus in dilute HCl digests fibrin with great readiness. From this it seems that the pylorus contains a substance which in the presence of the acid is transformed into pepsin, thus resembling the zymogen of the pancreas. The name, pepsinogen, has been suggested for this substance.

§ 19. *Brücke's Pepsin*.—Remove the mucous membrane of the stomach; wash it with cold water, cut into fine pieces; digest with dilute phosphoric acid at 38°. Filter; to the filtrate add clear calcium hydrate until a violet color is imparted to litmus paper. Collect the bulky precipitate of calcium phosphate which contains the pepsin. Wash carefully with a little cold water; suspend in water and dissolve by the addition of dilute hydrochloric acid, avoiding carefully excess of acid. Reprecipitate with calcium hydrate (much of the peptones which went down with the first precipitate, now remains in solution). Collect the precipitate; redissolve in dilute hydrochloric acid, and place the solution in a flask. Through a long funnel reaching to the bottom of the flask, add a saturated solution of cholesterin in a mixture of four parts of alcohol and one of ether. The cholesterin slowly rises to the surface, taking up the pepsin. Shake a few times; col-

lect the cholesterin on a filter : wash first with water acidulated with acetic acid, then with pure water, until the filtrate is no longer rendered turbid on the addition of silver nitrate. Transfer the cholesterin, while still moist, to a flask. Shake with ether which is entirely free from alcohol. The ether dissolves the cholesterin and rising to the top leaves a watery stratum below. Remove the ethereal layer ; add fresh ether and repeat until all the cholesterin has been removed. Filter the watery substratum which contains the pepsin and, when acidified, digests fibrin readily. The aqueous solution of pepsin may be still further purified by dialysis, as the pepsin will not pass through dialysis paper.

§ 20. *Wittich's Precipitation of Pepsin.*—Von Wittich first introduced the following method of removing pepsin from the mucus of the stomach : Wash the mucous membrane gently with water, cut into fine pieces and cover with alcohol. Allow the pieces to remain in the alcohol until they partially harden ; then pour off the alcohol ; dry the pieces of membrane by pressure between folds of blotting paper ; pulverize them ; cover with glycerin and allow to stand from one to two weeks. Filter the glycerin and add to the filtrate a large excess of absolute alcohol, when a flocculent precipitate containing impure pepsin falls. Filter and dissolve the residue on the filter with dilute hydrochloric acid (made by adding 20 c. c. of hydrochloric acid to 980 c. c. of distilled water). This solution contains no albuminous substances, and digests fibrin rapidly.

Pepsin, as prepared above, is a dirty white powder, soluble in water, glycerin and dilute acids, insoluble in alcohol and ether. The aqueous solution of pepsin does not pass through animal membranes, is not precipitated by nitric acid, or by acetic acid and potassium ferro-cyanide, but is precipitated by lead acetate. The aqueous solution of pepsin is, when it contains no free acid, without action upon fibrin, but when fibrin is added to such a solution, a part of the pepsin is taken up and held mechanically by the fibrin. From this combination pepsin cannot be removed by water nor glycerin, but it is readily

set free by a two per cent. solution of hydrochloric acid. Pepsin, in dilute hydrochloric, nitric or lactic acid, digests fibrin. This reaction goes on most rapidly at a temperature of about 40°. Dry pepsin can be heated to 100° without decomposition, and after having been subjected to that temperature, may be dissolved, and be found to digest albumen; but if a solution of pepsin be heated to 100°, the ferment is decomposed and does not regain its original properties on cooling. At the freezing point, the gastric juice of mammals is inert. If bile be added to the gastric secretion, the pepsin of the latter is carried down mechanically with the precipitate formed by the action of glycocholic acid upon the products of digestion. Moreover the alkalinity of the bile is sufficient to arrest gastric digestion.

§ 21. *Digestion a Chemical Process.*—The solution which results from peptic digestion contains the products of chemical change. The product of complete stomachic digestion is pepton; but ordinarily there is in the solution with the pepton a substance which is precipitated on neutralization and which is called parapepton. Coagulate some egg albumen neutralized with acid at 100°. Place the flocculent precipitate in good gastric juice containing .1 per cent. of HCl and leave at the temperature of the air. A small quantity of this filtered, will give a heavy precipitate on neutralization with potassium hydrate. Now continue the digestion at blood heat and from time to time neutralize a filtered portion. The neutralization precipitate will constantly grow smaller and after a few hours will no longer appear. Parapepton is the first product of gastric digestion, and it will remain longer in a juice poor in pepsin than in one rich in the same constituent. Parapepton is probably identical with the product obtained by the action of dilute acid alone on fibrin. Digest fresh blood-fibrin with a .1 per cent. solution of HCl for twenty-four hours; filter and neutralize, when parapepton or acidalbumen will be precipitated. The parapepton formed from boiled and that from raw fibrin differ somewhat. Digest with two portions of the same juice some raw and boiled fibrin. Filtered portions from both will

give precipitates on being boiled, but only that from the raw fibrin will give a coagulum on being heated.

*Pepton.*—Since pepton can neither be crystalized nor distilled it is very difficult to obtain it in the pure state. In preparing it the greatest care must be used; wash the fibrin with water, alcohol, ether and dilute acid. Prepare the pepsin according to Brücke's method. Render the digestion as complete as possible. Neutralize with  $\text{CaCO}_3$  or  $\text{Na}_2\text{CO}_3$ . Place the neutralized solution in a dialyser, and change the water frequently. Pepton in a neutral solution does not pass through animal membranes rapidly and may be left on the dialyser twenty-four hours. Remove the pepton solution from the dialyser, evaporate it to a syrup, precipitate the pepton by the addition of strong alcohol. Collect the precipitate, redissolve in water and reprecipitate with alcohol. Repeat the fractional precipitation several times. In this way a pepton of constant composition can be obtained.

Pepton is readily soluble in water. The solution is covered with a pellicle on evaporation and forms a syrup which is thick at a low, thin at a higher temperature. Pepton gives the following reactions, all of which, except the first, are common to all albuminous substances: (1) A dilute aqueous solution with potassium or sodium hydrate and a few drops of dilute solution of copper sulphate gives, on being warmed, a beautiful rose color, while other albuminous substances, treated in the same way, give a blue or violet-colored solution. (2) Heated with strong nitric acid it forms a dark-yellow colored fluid. (3) Dissolved in acetic acid, then treated with concentrated sulphuric acid, it forms a beautiful violet, feebly fluorescent solution, which after due concentration gives, on spectroscopic examination, a band similar to that of hydrobilirubin. (4) It gives a red coloration on being heated with Millon's reagent.\*

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\* Millon's reagent is prepared as follows: Dissolve some metallic mercury in an equal weight of strong nitric acid, first in the cold and then at a gentle heat. As soon as the metal is dissolved, add two volumes of water to one of the nitric acid solution. Allow to stand for several hours and pour off the clear fluid from the crystalline deposit. This fluid on being heated with any albuminous substance gives a red coloration.

When an aqueous solution of pepton is evaporated to dryness it leaves a whitish-yellow, gummy mass, which is very hygroscopic. From neutral aqueous solution it is precipitated by alcohol; but is not precipitated by heat.

§ 21. *Examination of Vomited Matters.*—For diagnostic purposes, the physician often desires to know whether the gastric juice, as obtained from vomited matter, is capable of performing its physiological duties; this may be done as follows: The substances under examination, if not sufficiently liquid, are stirred with water and filtered. To a portion of the clear filtrate, a piece of fibrin, prepared from blood and well washed, is added, and the whole is kept at about 40° in an air-bath for twelve hours. If at the expiration of this time the fibrin has not been perceptibly dissolved, or if putrefaction, as manifested by the odor, has begun, the gastric juice contained in the vomited matters is inert. This want of activity may be due to the absence or paucity of either the pepsin or free normal acid.

To another portion of the clear filtrate add an equal volume of a one-tenth per cent. solution of hydrochloric acid. To this add a small piece of fibrin, and treat as above. If now the digestive action proceeds normally, the physician recognizes the fact that the indigestion of his patient is due to an insufficient supply of the normal acid; while if the fibrin remains insoluble the pepsin is deficient.

If the piece of fibrin be dried and weighed before being added to the solution and the fibrin remaining at the expiration of the twelve hours be also dried and weighed, the exact degree of action may be ascertained.

Grünhagen has introduced the following method of approximately estimating the amount of pepsin: Some washed fibrin is covered with a .2 per cent. solution of hydrochloric acid and allowed to stand at the ordinary temperature for an hour or two. The jelly-like mass of fibrin is then freed from the dilute acid by pressure and the solid cake is placed on a filter. The funnel supporting the filter is set in a beaker which is placed in an air-bath with the temperature at 40°. The fluid to be tested is now poured upon the fibrin and the

amount of pepsin is estimated from the rapidity with which the fibrin is dissolved and the solution passes through the filter.

Grützner's method is as follows: Finely-divided fibrin is covered with a dilute ammoniacal solution of carmin for about twenty hours. The fibrin is then removed, washed with water, and then allowed to stand in a .2 per cent. solution of HCl until it forms a jelly-like mass. The fibrin now having a uniform rose color is subjected to the action of the fluid under examination. The rapidity of digestion is indicated by the depth of color imparted to the fluid. For comparison a scale of colors of ten shades is made as follows: An ammoniacal solution of carmin is mixed with glycerin in such a proportion that it will contain .1 per cent. of carmin. This is diluted with water in ten different proportions, so that No. 1 shall contain 19.9 c. c. of water and .1 c. c. of the glycerin-carmin solution. No. 2 contains 19.8 c. c. of water and .2 c. c. of the carmin. No. 5 contains 19.5 c. c. of the former and .5 c. c. of the latter, etc.

In uremia, vomited matters often contain urea, ammonium carbonate and bile. The urea is detected by concentrating the filtrate on the water-bath to a small volume and then adding nitric acid, when on standing crystals of urea nitrate form. Bile-acids and bile-pigments are detected respectively by Pettenkoffer's and Gmelin's tests. (See under bile).

The vomit of persons suffering with cholera is alkaline in reaction, is turbid from the presence of mucus and epithelium, contains albumen, and frequently urea.

Vomit produced mechanically from an empty stomach is neutral or alkaline, and consists principally of mucus. The same condition has been observed in the vomit of hysterical persons and of those addicted to the excessive use of alcoholic drinks.

In hæmatemesis, the blood, unless excessive in quantity, is disintegrated by the action of the acid of the stomach, the hæmaglobin being converted into hæmatin. This may be dissolved in an alkali and examined with a spectroscope.

## ANALYSIS OF BILE.

§ 22. Bile may be obtained for analysis after the death of the animal, or during life by means of a biliary fistula. Fresh bile can be procured also by putting an animal under the influence of chloroform, laying open the abdomen and drawing the bile from the gall-bladder with a trochar, aspirator, or hypodermic syringe. It is very seldom that an opportunity is presented of making an analysis of the bile of a living man. Consequently an examination of this secretion is not undertaken as an aid to treatment in the individual case. But we are prompted in this work by the following considerations: (1) We hope to understand more fully its chemical composition and physiological action; (2) an obscure case which has terminated fatally may present an abnormal condition of this secretion. Thus, by studying the dead, we may be better prepared to protect the living.

In man, and most other omnivorous and carnivorous animals, bile is of a yellow, reddish-yellow, or brownish-yellow color; the principal coloring ingredient being bilirubin. In the herbivora, the bile has a green or brown color, which is due to the presence of biliverdin. As taken from the gall-bladder, this secretion contains more or less mucus, and is neutral or feebly alkaline in reaction. I once found that the bile taken from a patient who died of peritonitis from cancer of the rectum and colon, was strongly acid. In order to ascertain the reaction, the bile should be diluted with distilled water, filtered and tested with litmus paper. The inorganic constituents of bile are sodium chloride, calcium phosphate, sodium phosphate, oxide of iron, and traces of copper. The organic are mucin, cholesterin, lecithin, oil, bile-pigment, and bile-acids. In the bile of man, glycocholic is the principal acid; while in the cat, taurocholic acid predominates; and in hogs hyoglycocholic acid is present. In all cases, these acids are combined with sodium or potassium.

§ 23. *Crystallized Bile-Acids*.—Place the bile in an evaporating dish on the water-bath, and concentrate to one-sixth its volume. To the residue, add absolute alcohol, stir and filter



through animal charcoal. Repeat this operation with several successive portions of alcohol. The alcoholic filtrate (which, if not perfectly colorless, should be again filtered through animal charcoal) is concentrated on the water-bath to one-quarter its volume and poured into a clean flask. To this add ether in great excess. The ether precipitates sodium taurocholate and glycocholate. The former of these only is present, if the bile used was that of the cat or dog. Place the flask containing the suspended precipitate in a freezing mixture and allow to stand for twenty-four hours. At the expiration of this time, (it will frequently require a longer time than twenty-four hours for crystallization) the precipitate will have formed in needle-shaped crystals. Decant the mixture of alcohol and ether. Wash the crystals by decantation with pure ether; dissolve them in water and use as a solution of purified bile.

§ 24. *Pettenkoffer's Test for Bile-Acids.*—To a dilute solution of purified bile in a test tube add a drop of a solution of cane sugar, and then sulphuric acid drop by drop; keeping the temperature as near 70° C. as possible by placing the tube in cold water if too warm, or by gently heating it if too cool. If bile-acids be present in the proportion of one part of the acids to five hundred of water, or in greater quantity, a white precipitate will appear upon the addition of the first drop of sulphuric acid. The addition of the acid must be continued until this precipitate is dissolved. The solution now takes a cherry-red color, becoming purple on standing. If only minute quantities of the bile-acids be present, the white precipitate does not appear, but the solution becomes colored as before. This solution generally has a characteristic purple foam, but if no foam exists, as is sometimes the case, it can be obtained by the addition of a few drops of sodium carbonate to the cherry-red solution. When this is done the froth produced by the liberated gas has a purple hue, which instantly disappears. The student should ascertain the limit of this test and the various shades of color according to the amount of bile present, by diluting his solution of purified bile with definite proportions of water. This test fails to reveal bile-

acids in the presence of alcohol, and oxidizing substances as nitrates and chlorates.

Dilute some crude bile with an equal volume of water, filter and apply Pettenkoffer's test. To some urine in one test tube and to some saliva in another, add bile, filter and test each as above.

To some urine add bile, filter and apply the following modification of Pettenkoffer's test: Render the urine alkaline with ammonium hydrate, then add lead acetate as long as a precipitate is formed; filter; dry the precipitate at  $100^{\circ}\text{C}$ .; remove it to a dish, add absolute alcohol and boil. Again filter and evaporate the alcoholic filtrate to dryness on the water-bath. Dissolve the residue in water, rendered slightly alkaline with sodium hydrate. To this solution apply the sugar and sulphuric acid as given above.

#### GLYCOCHOLIC ACID— $\text{C}_{26}\text{H}_{43}\text{NO}_6$ .

§ 25. This substance exists as a sodium salt in the bile of man and that of the ox. It is not present in the bile of the dog whether that animal be fed upon animal, vegetable, or mixed food. It is found in small quantities in the urine of jaundiced persons. Traces of this acid can generally be found in the fæces of oxen.

*Preparation.*—(1) To a solution of purified bile, add dilute sulphuric acid until a cloudiness appears and remains on being stirred or shaken. Care must be taken not to add enough sulphuric acid to redissolve this precipitate. The dilute acid combines with the base of the glycocholate of sodium and frees glycocholic acid. The latter, after standing some hours, crystallizes in needles. Collect the crystals on a filter, wash them with a little cold water, dissolve in absolute alcohol, reprecipitate by the addition of ether, collect and dry by pressing between folds of blotting paper.

(2) Gorup-Besanez employs the following method: Evaporate fresh ox-gall almost to dryness on the water-bath. Extract the residue with ninety per cent. alcohol. Distil or evaporate the alcohol, dissolve the residue in water, add milk of lime, warm and filter. To the filtrate, after cooling, add dilute sul-

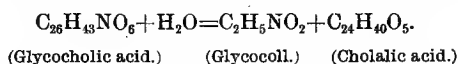
phuric acid until a cloudiness appears and remains (avoiding an excess of the acid). Allow to stand for some hours, when the glycocholic acid will have crystallized. Collect on the filter, wash with cold water, redissolve in much lime water, reprecipitate with dilute sulphuric acid as above, collect, and dry with blotting paper.

(3) Concentrate fresh ox-gall to half its volume. Place it in a tall graduated measure, add one-twentieth as much hydrochloric acid, then add ether (5 c. c. to 100 c. c. of the bile). The glycocholic acid is precipitated and after a few days crystallizes. Collect the crystals on the filter and wash with cold water until the filtrate is colorless. Dissolve the crystals as they remain on the filter, in hot water. As the filtrate cools, glycocholic acid recrystallizes. With some specimens this method furnishes very satisfactory results, while in other cases, without any apparent cause, it fails completely.

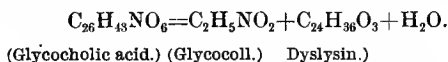
*General Properties.*—Glycocholic acid is soluble in 303 parts of cold, or 120 parts of hot water. From its solution in the latter it crystallizes, on cooling. It is freely soluble in absolute alcohol, insoluble in ether. If the alcoholic solution be evaporated, glycocholic acid remains as a hard, amorphous mass: while if water be first added to the alcoholic solution, the acid separates in fine crystals. Its solution colors blue litmus red. Glycocholic acid dissolves without decomposition in strong sulphuric, hydrochloric and acetic acids. It is freely soluble in the hydrated alkalis, forming glycocholates. When heated with an alkaline carbonate, carbonic acid is liberated. Its alkaline salts give the same reactions that the free acid does. Glycocholates of the alkalis, alkaline-earths and silver are soluble in water; while the combinations with other bases are insoluble or soluble with difficulty in water. All glycocholates are soluble in alcohol. Sodium, potassium and silver glycocholates form in needle-shaped crystals on the addition of ether to their alcoholic solutions, while the corresponding salts of barium and lead are amorphous. Neutral lead acetate precipitates glycocholic acid from aqueous solution; this precipitate being soluble in hot alcohol, and forming a flocculent deposit on cooling.

If glycocholic acid be dissolved in concentrated sulphuric, or hydrochloric acid and the solution warmed, *cholonic acid*,  $C_{26}H_{41}NO_5$ , separates on cooling. It will be seen, by comparison of the formulæ, that cholonic acid is formed from glycocholic acid by the abstraction from the latter of one molecule of water. Cholonic acid is insoluble in water, soluble in alcohol and is never crystalline. Its barium salt is insoluble in water and by this means is easily distinguished from glycocholic, also from cholalic acid. (Hoppe-Seyler).

If a solution of glycocholic acid be boiled with a caustic alkali, a saturated solution of barium hydrate, or dilute acids, it takes up a molecule of water and forms *glycocoll* and *cholalic acid*:



By further action of dilute acids on glycocholic acid, *dyslysin* is produced:



§ 26. Taurocholic, also known as choleinic acid, is found together with glycocholic acid in human and ox bile. It will be seen from its formula that this acid contains sulphur, which exists also in *hyotaurocholic* and *chenotaurocholic* acids.

*Preparation.*—Taurocholic acid is best obtained from the bile of the dog, in which it is the only acid. Evaporate dog-bile to dryness on the water-bath. Extract the residue with hot alcohol and filter through animal charcoal. Evaporate the filtrate on the water-bath, dissolve the residue in a small quantity of absolute alcohol. Precipitate the taurocholate of sodium from this alcoholic solution by the addition of ether. Dissolve the precipitate in water, render the solution alkaline with ammonia, and reprecipitate with the basic acetate of lead. Wash the precipitated taurocholate of lead with water, dissolve in hot alcohol and filter before allowing to cool. Treat the filtrate with a stream of hydrosulphuric acid gas, and remove the sulphide of lead by filtration. Concentrate the filtrate and

precipitate by the addition of ether. Taurocholic acid forms first in an amorphous mass, then in fine crystals, which on exposure to the air dissolve into a syrup.

*Properties and Decomposition.*—Taurocholic acid gives a decidedly acid reaction, is soluble in water and alcohol, insoluble in ether. In both the free and combined state, it is easily decomposed. By the evaporation of the aqueous solution of the free acid, its molecules are broken up, forming *taurin* and *cholic acid*, in the same manner that glycocholic acid is with much greater difficulty changed into glycoll and cholic acid. The same decomposition is produced by the action of alkalis and dilute acids, and also occurs normally in the intestines. This acid, as well as glycocholic, forms salts with vegetable alkaloids.

*Separation and Estimation.*—Taurocholic is separated from glycocholic and cholic acids by means of the different reactions of these substances with neutral acetate of lead. This reagent completely precipitates glycocholic and cholic acids from aqueous solutions of their salts; but throws down only a trace of taurocholic acid unless the solution be strongly alkaline. Thus, if to a solution of purified ox-bile, prepared as directed under crystallized bile, neutral acetate of lead be added, the glycocholate of lead is precipitated and may be removed by filtration. If now to the filtrate, basic acetate of lead or the neutral acetate with ammonia be added, the taurocholate of lead is precipitated. From this precipitate the taurocholic acid may be separated by the following method: Place the lead-precipitate in an evaporating dish, add a solution of carbonate of soda and evaporate to dryness on the water-bath. Extract the residue with hot absolute alcohol and filter. Concentrate the filtrate and precipitate the taurocholate of soda by the addition of ether. If the taurocholate of soda, thus obtained, be transferred to a platinum dish, nitrate of potash be added, the whole burnt until all the organic matter is destroyed, the sulphuric acid of the ash estimated in the usual way with baric chloride, the amount of taurocholic acid may be ascertained. From the amount of sulphuric acid, calculate

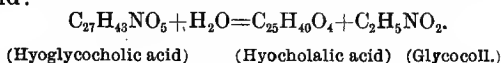
the sulphur, one part of which represents 16.28 parts of taurocholic acid.

HYOGLYCOCHOLIC ACID,  $-C_{27}H_{43}NO_5$ .

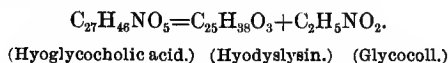
§ 27. *Preparation*.—This acid, known also as hyocholic, has been found as yet only in the bile of the pig. The bile of this animal is decolorized by agitation with animal charcoal and filtration. To the filtrate, crystallized sodium sulphate is added to saturation. This precipitates the bile-acid which is now collected upon the filter, washed with a saturated solution of sodium sulphate, and then dissolved in water. From the aqueous solution of this compound the free acid is precipitated by the addition of hydrochloric acid and collected upon the filter paper.

*Properties*.—As thus prepared, hyoglycocholic acid is a colorless, amorphous mass, insoluble in water, slightly soluble in ether, and freely soluble in absolute alcohol. Its solution has an acid reaction and a bitter taste. It combines with bases forming salts. Its alkaline salts are freely soluble in water; while the corresponding salts of the alkaline-earths and metals are insoluble in the same menstruum. All its salts are soluble in alcohol. From aqueous solutions of its salts, the acid is precipitated by the addition of alkaline sulphates or chlorides.

*Decomposition*.—On being boiled with hydrated alkalis or with dilute acids, the atoms of the hyoglycocholic acid molecule are rearranged forming *glycocol* and *hyocholalic acid*. This change, as will be seen from the equation, corresponds to that by which glycocol and cholalic acid are formed from glycocholic acid:



On further boiling with dilute hydrochloric acid, *hyodyslysin* is produced:



HYOTAUROCHOLIC ACID,  $-C_{27}H_{45}NSO_5$ .

§ 28. This acid, which is identical with Strecker's hyocholeinic, exists in minute quantities with hyoglycocholic acid in

the bile of the pig. It has never been obtained in the pure state. It will be seen from comparison of the formulæ that the molecule of hyotaurocholic acid contains one atom of carbon more and one of oxygen less than the taurocholic acid molecule. The same relation exists between hyoglycocholic and glycocholic acids. By long boiling with alkalis or dilute acids, hyotaurocholic acid is converted into *taurin* and *hyocholalic acid*.

CHENOTAUROCHOLIC ACID,— $C_{29}H_{49}NS_6$ .

§ 29. *Preparation*.—This acid is found in the bile of the goose. The gall of this bird is evaporated, the residue extracted with absolute alcohol, the solution decolorized with animal charcoal and filtered, and the filtrate treated with ether. The pasty mass precipitated by the ether is collected on the filter, washed with a saturated solution of sodic sulphate and dissolved in absolute alcohol. After the addition, to the solution, of ether and after standing, chenotaurocholate of sodium is deposited in a crystalline mass. This is dissolved in water and treated with basic acetate of lead. The lead precipitate is suspended in alcohol and treated with hydrosulphuric acid gas. The sulphide of lead is removed by filtration and the filtrate evaporated, when an amorphous mass of chenotaurocholic acid remains.

*Properties*.—This acid, prepared as above, is soluble in water and in alcohol. It combines with the alkalis forming salts. From aqueous solution of its alkaline salts, the acid is liberated by the addition of hydrochloric and other mineral acids. The chenotaurocholates of lead, silver and barium are insoluble in water. On being heated for a long while with a saturated solution of baric hydrate, chenotaurocholic acid is decomposed with the formation of *taurin* and *chenocholalic acid*,  $C_{27}H_{44}O_4$ .

CHOLALIC ACID,— $C_{24}H_{40}O_5$ .

§ 30. This acid is by some authors known as *cholic acid*. (See Burdon-Sanderson's Handbook for the Physiological Laboratory, p. 504.) But the name, cholic acid, has been used to designate different substances, and consequently is now quite

worthless. For instance, the cholic acid of Gmelin and Strecker is identical with the glycocholic acid of other chemists, while the cholic acid of Demarcay and Berzelius is identical with the cholalic acid of Strecker.

*Occurrence.*—Cholalic acid combined with glycocoll forms glycocholic acid; combined with taurin, forms taurocholic acid. Cholalic acid exists free in the intestines and in the excrement of man and the domestic animals. It is also often found in the urine. By spontaneous decomposition of bile, this acid is set free from its combinations with taurin and glycocoll.

*Preparation.*—(1) Boil ox, or dog-bile for twenty-four hours, or until no ammonia is given off, with a solution of barium hydrate saturated at the boiling point. (Adding water whenever necessary to prevent evaporation to dryness.) Add to the mixture hydrochloric acid and collect the precipitate, which forms, upon the filter. Wash with water, dissolve in sodium hydrate, avoiding an excess of the solvent, add ether and hydrochloric acid, and set aside for a few days. During this time impure cholalic acid is deposited in crystals. Collect the crystals, press them between folds of blotting paper, redissolve in a little hot alcohol and add water to the alcoholic solution until a cloudiness appears. Allow to stand for some days, when cholalic acid forms in tetrahedrons.

(2) Hofmann gives the following method for preparing cholalic acid: Dissolve crystallized bile in a concentrated solution of potassium hydrate. Heat this solution in hermetically sealed tubes at 100° C. for twenty-four hours, and then at 120° for one hour. Treat the contents of the tubes with water to which a little ether has been added, then add hydrochloric acid. Pure cholalic acid is deposited on standing.

*Properties.*—Cholalic acid appears both in an amorphous and a crystalline condition: If a solution of the amorphous variety in ether be concentrated, the acid forms in four-sided prisms, the ends of which terminate in two oblique faces. From its saturated solution in hot alcohol, cholalic acid forms on cooling in modified octohedrons or tetrahedrons. The crystals are permanent in the air, slightly soluble in ether,



freely soluble in hot alcohol, and insoluble in water. They are brittle, opaque, and colorless. The amorphous acid has a waxy appearance and a doughy consistency. Solutions of free cholalic acid have an acid reaction and a bitter taste. The acid is freely soluble in alkalis and on being heated with an alkaline carbonate liberates carbonic acid.

*Compounds.*—The alkaline salts are sparingly soluble in hot alcohol and freely soluble in water. The barium salt crystallizes in fine needles, is freely soluble in alcohol, soluble in 30 parts of cold, or 23 parts of hot water. From aqueous solutions of its barium and alkaline salts, the acid is precipitated by the addition of silver nitrate, or lead acetate. The cholalates of lead and silver are soluble in hot alcohol. If cholalate of silver be dried at the temperature of the water-bath and then gently warmed with iodide of methyl, the cholalate of methyl  $(\text{CH}_3)\text{C}_{24}\text{H}_{39}\text{O}_5$  is formed, and may be obtained in the pure state by treating the mass with potassium hydrate solution, filtering, adding ether to the filtrate and allowing to stand. The cholalate of methyl crystallizes in long colorless prisms. The cholalate of ethyl  $(\text{C}_2\text{H}_5)\text{C}_{24}\text{H}_{39}\text{O}_5$  is formed when an alcoholic solution of cholalic acid is saturated with hydrochloric acid gas, allowed to stand for four hours, precipitated by the addition of water, the precipitate shaken with ether and sodium hydrate. As the ether evaporates ethyl cholalate is deposited in a crystalline mass, which may be collected, washed with water, dissolved in absolute alcohol and reprecipitated by the addition of ether.

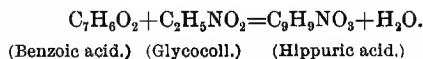
Cholalic acid heated to  $200^\circ$  with glycerin produces a mixture of glycerides. If ammonium cholalate (prepared by treating an alcoholic solution of cholalic acid with ammonia gas and adding ether) be heated on the oil-bath until no water is given off, cholamid  $(\text{NH}_2)\text{C}_{24}\text{H}_{39}\text{O}_4$ , remains. If dry cholalic acid be heated, an aromatic substance is given off.

*Occurrence in Urine and Fæces.*—Cholalic acid is often the only bile-acid present in abnormal urine, the glycocholic and taurocholic acids having been decomposed either in the body, or in the urine after emission. Even when no traces of bile-

acids can be detected in the urine, cholalic acid in quantity may be obtained from the fæces. For this purpose Hoppe-Seyler recommends the following process: The fæces are extracted with alcohol and the extract filtered. To the filtrate add acetic acid and concentrate to a syrup on the water-bath. The syrup is washed with cold water. To the part insoluble in cold water, add barium hydrate and warm water. Treat this solution with carbonic acid gas until it becomes neutral in reaction, avoiding an excess of carbonic acid. Heat the mixture and filter while boiling. Wash the residue on the filter with hot water as long as any is dissolved. Unite the filtrate and wash water, and concentrate to a small volume. After cooling, add ether, then hydrochloric acid; agitate, then allow to stand for a short time. Cholalic acid, which is now deposited, is collected on a filter, washed with cold water and dissolved in absolute alcohol. The alcoholic solution is decolorized, if necessary, with animal charcoal, concentrated to a small volume and allowed to stand until the cholalic acid crystallizes. That the crystals are cholalic acid may be demonstrated by applying Pettenkoffer's test for bile-acids, by heating some of the crystals, and observing the peculiar aromatic odor produced, and by their dextrorotatory effect upon polarized light.

GLYCOCOLL (AMIDO-ACETIC ACID),— $C_2H_5NO_2$ .

§ 31. *Occurrence*.—In the bile, glycocoll is combined with cholalic and hyocholalic acids forming glycocholic and hyoglycocholic acids. When the bile-acids are decomposed in the intestines and a part, if not all, of the cholalic acid passes out in the fæces, it is supposed that the glycocoll and taurin are returned to the blood. In what condition, whether free or combined, the glycocoll is taken into the circulation is not known. Some glycocoll is constantly excreted in the urine as hippuric acid, which is formed in the body by the combination of glycocoll and benzoic acid:

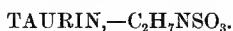


*Preparation*.—Boil hippuric acid for an hour with hydrochloric acid, dilute and set aside. On cooling, benzoic acid is

deposited in crystals. The supernatant fluid is decanted and evaporated on the water-bath to dryness. The residue is dissolved in a little water and filtered. The filtrate is boiled for five minutes with hydrated oxide of lead, then treated with a current of hydrosulphuric acid gas and filtered. The filtrate is concentrated on the water-bath until crystals of glycocoll are deposited.

*Properties.*—The crystals are generally four-sided prisms, soluble in cold water, insoluble in ether and alcohol. The solution of glycocoll has an acid reaction and a sweet taste. If recently precipitated hydrated oxide of copper be boiled with an aqueous solution of glycocoll the former is dissolved. On evaporating the blue solution, thus obtained, the double oxide of copper and glycocoll is deposited in needle-shaped crystals.

If a solution of glycocoll be treated with nitrous acid, the former is converted into glycollic acid, water and nitrogen gas.



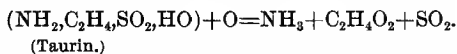
§ 32. From its composition, especially from the fact that it contains sulphur in complex combination with carbon, nitrogen, hydrogen, and oxygen, it is quite evident that taurin is derived from albumen. In the bile as contained in the gall-bladder, taurin is combined with cholalic acid. And, like glycocoll, it is absorbed from the intestines. Taurin has been found in the muscles and the lung substance of animals (Hoppe-Seyler). The sulphur of this substance is oxidized and appears in the urine as sulphuric acid.

*Preparation.*—Boil the bile of the dog or of the ox for three hours with dilute hydrochloric acid. Filter, and evaporate the filtrate to dryness on the water-bath. Extract the residue with absolute alcohol. Treat the part insoluble in alcohol with water, filter the aqueous extract. Concentrate the filtrate and allow to cool when taurin crystallizes. In order to purify the taurin, dissolve the crystals in dilute spirits, add lead acetate, treat with hydrosulphuric acid gas, and filter. Evaporate the filtrate, wash the residue with absolute alcohol. Dissolve the part insoluble in alcohol in water, filter the aqueous solution, concentrate and allow to cool, when pure taurin crystallizes.

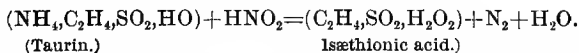
*Properties.*—The crystals are often very large, colorless, four or six-sided prisms. They are insoluble in absolute alcohol, soluble in dilute spirits, soluble in both cold and hot water, insoluble in ether. The crystals are not decomposed on being boiled with dilute acids or alkalis. On being heated above 240° they swell and then burn, giving off sulphurous acid gas and leaving a residue. The aqueous solution is not precipitated by acids, alkalis nor salts. If taurin be treated with phenol and sodium hypochlorite a bluish-green color is produced.

If a solution of taurin be boiled with calcium chloride and the mixture evaporated, fine needle-shaped crystals of  $\text{Ca}(\text{C}_2\text{H}_6\text{NSO}_3)_2$  will remain. By spontaneous evaporation of a solution of taurin to which silver oxide has been added, tablets of  $\text{Ag}(\text{C}_2\text{H}_6\text{NSO}_3)$  appear. These blacken on exposure to the air. They are soluble in water, insoluble in ether. Heat recently precipitated oxide of mercury on the water-bath with an excess of a solution of taurin, until the yellow color disappears and a white precipitate remains. Allow to stand for an hour, collect the white precipitate on a filter, and wash with hot water. The compound  $\text{Hg}(\text{C}_2\text{H}_6\text{NSO}_3)_2 + \text{H}_2\text{O}$ , remains on the filter, insoluble in water. It can be heated to 140° without decomposition. When greater heat is applied the mercury is driven off and a black residue remains. (Hofmann.)

If a solution of taurin in potassium hydrate be boiled for some time, ammonia is given off and sulphurous and acetic acids are formed :



If a solution of taurin be treated with nitrous acid and boiled, or if crystals of taurin be dissolved in dilute nitric acid, potassium nitrate added and the whole gently warmed, isæthionic acid, water and nitrogen are formed :



That taurin is ammonium isæthionate is proven by synthesis as represented in the following equation :



wash with cold alcohol and water, redissolve in boiling alcohol, from which pure cholesterin is deposited on cooling.

*Properties.*—Cholesterin crystallizes in two forms. From its solution in chloroform, or benzol, it forms in fine, colorless, needle-shaped crystals. This form contains no water of crystallization. From its solution in boiling alcohol, cholesterin is deposited in large rhombic tablets. These contain water and are represented by the formula  $C_{26}H_{44}O + H_2O$ . The tablets generally have a characteristic notch in one corner. Cholesterin is soluble in boiling alcohol, in hot ether, in chloroform, in benzol, in hot glycerin and in solutions of the alkaline glycolates, taurocholates, and cholalates. It is insoluble in water, cold alcohol, dilute acids and strong alkalis.

*Tests.*—(1) Place some cholesterin on a glass slide and add a few drops of concentrated sulphuric acid. A beautiful red color is produced, which, on the addition of water, becomes green. If the crystals of cholesterin be treated with a drop of sulphuric acid and then a little iodine be added, a series of colors, violet, blue, green, red, yellow and brown, is developed. These changes are best observed through a microscope of low power.

(2) Dissolve crystals of cholesterin in chloroform and add an equal volume of concentrated sulphuric acid. The solution becomes violet, then blue, green, and finally yellow. The addition of water decolorizes the solution instantly.

(3) To cholesterin crystals in an evaporating dish, add a few drops of nitric acid and gently heat to dryness. A yellow residue remains, which on the addition of ammonia becomes red. (But is not changed by sodium hydrate, and thus is distinguished from the murexid test.) (Schiff's test.)

(4) To hydrochloric acid add ferric chloride, pour the mixture upon crystals of cholesterin in a porcelain dish and heat. The cholesterin is colored red, then violet, and finally blue.

(5) Dissolve cholesterin in acetic acid by the application of heat. Allow to cool, when needle-shaped crystals of cholesterin acetate  $C_{26}H_{44}O, C_2H_4O_2$ , are deposited. Treat the crystals with

alcohol, when cholesterin will be set free and form in tablets. (Hoppe-Seyler.)

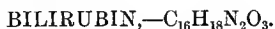
Cholesterin is recognized and distinguished from other substances by its crystalline forms and by its reactions with sulphuric acid and with sulphuric acid and iodine.

#### BILE-PIGMENTS.

§ 34. Besides the acids, which have been described, there are certain coloring matters which characterize the biliary secretion. In different animals, these coloring principles vary. Thus, in the bile of the ox, biliverdin predominates; while the color of human bile is principally due to the presence of bilirubin. One of these coloring matters can be changed into another by means of oxidizing or reducing agents. Upon this fact depend the various tests for bile-pigment.

*Gmelin's Test for Bile-Pigment.*—Dilute some bile and spread upon the bottom of a white dish, and add a few drops of fuming nitric acid. A precipitate will form around each drop, and then a green, blue, red, and yellow coloration will extend outward in rings, due to the action of the strong acid upon the coloring matter of the bile. If fuming nitric acid be not at hand, a substitute may be found by adding some strong sulphuric acid to the ordinary nitric acid, and allowing the mixture to stand in the sunlight until red fumes appear. This test is more distinct with human or dog bile than with that of the ox.

Hoppe-Seyler applies Gmelin's test in the following manner: Pour into a test tube some fuming nitric acid. Carefully add, without agitating, dilute bile or the fluid suspected to contain bile. Allow to stand and observe from time to time. Soon colored rings appear. These are, from above downwards, green, blue, violet, red and yellow.



§ 35. *Preparation.*—Bilirubin exists free in small quantities in the bile of man and of the carnivora. It is found also in the gall-stones of both men and oxen. Pulverize human gall-stones; wash the powder with boiling water to remove any trace of bile acids; then with ether to dissolve the fat; next

with dilute hydrochloric acid in order to remove any inorganic matter, especially the salts of calcium; and at last with hot water. Dry the residue on the water-bath and boil it with chloroform. Filter and distil the chloroform from the filtrate. Wash the residue with absolute alcohol, which removes the bilifuscin. The bilirubin remains as a residue, insoluble in absolute alcohol. Dissolve this in a little hot chloroform and concentrate the solution until bilirubin begins to separate as an orange-red powder, then add alcohol which completes the precipitation.

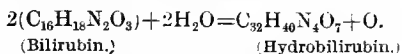
Shake the bile of man with chloroform; draw off the chloroform by means of a pipette; evaporate the chloroform, and wash the residue with alcohol and then with ether, when bilirubin remains.

*Properties.*—If a chloroform solution of bilirubin be allowed to evaporate spontaneously, the substance is deposited in rhombic tablets. But if the chloroform be evaporated, even at the temperature of the water-bath, bilirubin remains, as an amorphous powder. It is insoluble in water, ether and absolute alcohol. Soluble in chloroform, benzol, amylic alcohol and glycerin. From its solution in the alkalis, it is precipitated unchanged on the addition of dilute acids. Its alkaline solutions have a yellow color, which is observable even after dilution with many thousand times their volume of water. These dilute solutions respond to Gmelin's test, or the play of colors is produced by the tincture of iodine, by bromine or by ozone.

Bilirubin combines with many bases. The calcium compound is formed by precipitating the bilirubin from its solution in ammonia. This precipitate, dried in a vacuum over sulphuric acid, is a brownish powder, insoluble in water, alcohol, chloroform, or ether. Bilirubin is precipitated from its solution in ammonia also by barium chloride, silver nitrate and lead acetate. If a solution of bilirubin in sodium hydrate be left in an evaporating dish for a few days, the solution becomes green. Oxygen has been absorbed and the bilirubin has been converted into biliverdin. On the other hand, by the action of



reducing agents, as sodium amalgam, bilirubin is deoxidized with the formation of *hydrobilirubin*:



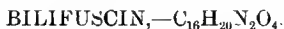
(Bilirubin.)

(Hydrobilirubin.)



§ 36. In Gmelin's test for bile-pigment, the first color in the series, that is developed, is green. This is due to the oxidation of bilirubin into biliverdin. The latter exists preformed in the bile of the ox and many other animals; but it is generally prepared from bilirubin.

Expose a solution of bilirubin in sodium hydrate to the air in shallow dishes until the yellow color of the solution is transformed into green. Then add hydrochloric acid, which precipitates the biliverdin. Collect the precipitate on the filter and wash with water until the filtrate ceases to give a test for chlorides; then dissolve in alcohol and evaporate. Biliverdin remains as a dark-green, amorphous powder. Biliverdin is insoluble in water, chloroform and ether; soluble in alcohol and alkalis. The solutions have a green or bluish-green tint. On the application of fuming nitric acid to alkaline solutions of biliverdin, a series of colors, blue, violet, red and yellow, is observed. If an alkaline solution of this coloring principle be warmed and treated with sulphurous acid gas, the green color of the solution is changed to yellow. By the action of the reducing agent, the biliverdin has probably been reduced to bilirubin.



§ 37. Wash pulverized human gall-stones with dilute hydrochloric acid, which dissolves the lime; then with water; next with ether, which removes the fat; finally boil with absolute alcohol, which dissolves the bilifuscin. Filter, evaporate the filtrate to dryness on the water-bath, wash the residue with water, then with ether and chloroform. Redissolve in a little absolute alcohol and evaporate, when bilifuscin, as a glistening, black residue, is observed.

Bilifuscin is insoluble in water and ether; slightly soluble in chloroform, and freely soluble in absolute alcohol. The alcoholic solution is of a yellow color; while the alkaline solutions

are reddish-brown. From its alkaline solutions, bilifuscin is precipitated as a brown powder on the addition of dilute hydrochloric acid. Bilifuscin is precipitated from its solution in ammonia by calcium or barium chloride.

When perfectly free from bilirubin, bilifuscin does not give Gmelin's reaction.

BILIPRASIN,  $\text{—C}_{16}\text{H}_{22}\text{N}_2\text{O}_6$ .

§ 38. Wash pulverized human gall-stones successively with ether, chloroform, dilute hydrochloric acid and water. Treat the residue insoluble in the above reagents with alcohol. Filter, and evaporate to dryness the alcoholic solution. Wash this residue with ether and chloroform. Redissolve in cold alcohol, filter and evaporate, when biliprasin remains.

Biliprasin, as obtained above, is a black, brittle mass which on being pulverized forms a dark-green powder. It is insoluble in water, ether and chloroform; freely soluble in spirits of wine. The alcoholic solution is of a beautiful green tint, which on the addition of an alkali is changed to brown. The brown color of the solution in ammonia enables one to distinguish between biliprasin and bilirubin; for the solution of the latter in ammonia is yellow. From its alkaline solutions, biliprasin is deposited as a green precipitate upon the addition of hydrochloric acid.

#### ORIGIN AND DESTINATION OF THE BILE CONSTITUENTS.

Sec. 38½. The bile acids and pigments are formed in the liver cells. The glycocholic and taurocholic acids are formed from the albuminous constituents of the blood; while the cholalic acid is *probably* formed from fat. After the bile passes into the intestines, the glycocholic and taurocholic acids are split up, the former into glycocholic and cholalic acid and the latter into taurin and cholalic acid. The cholalic acid wholly or in greater part passes out with the fæces; while the taurin and glycocholic are reabsorbed. It may be that portions of the glycocholic and taurin return to the liver and again combine with the acid. Be this as it may, some of the glycocholic passes to the kidney, there combines with benzoic acid and is excreted as hippuric acid; while the taurin is oxidized and its sulphur excreted through the kidney as sulphuric acid.

The bile-pigments are formed in the liver from the coloring matter of the blood. In the intestines they take up the free hydrogen, which is liberated by the process of fermentation constantly going on there, and are converted into hydrobilirubin (urobilin), which is one of the coloring matters of the urine.

#### BILIARY CALCULI.

§ 39 Gall-stones vary in size from those just visible to the unaided eye to those which are as large as an English walnut; and from a single one to seven thousand have been found in one person. Human gall-stones consist principally of cholesterin, with bile-pigments and salts of calcium. They contain generally as much as ninety-five per cent. of cholesterin. They are of a brownish color, and round or polyhedral with the faces worn smooth by the constant rubbing of the stones together in the bladder. When first removed, the gall-stones are generally soft and can be crumbled by pressure between the fingers, but on exposure to the air they harden. A nucleus of deeply colored matter, bile-pigment combined with lime, is found in many gall-stones. In others, the nuclei consist of mucus. Rarely one meets with white calculi of pure cholesterin. Again masses of crystalline cholesterin have been found in the gall-bladder. Frequently small, black or deep-red stones, known as pigment calculi, are found in the gall-bladder of the ox, very rarely in that of man. These are poor in cholesterin, rich in bile-pigment combined with lime, and contain traces of copper.

A quantitative analysis of biliary calculi is made as follows: Pulverize the stone, and weigh the powder; wash with boiling water, which removes traces of bile-acids; then treat the powder with a boiling mixture of equal parts of alcohol and ether and filter while boiling. As this filtrate cools, cholesterin is deposited; collect on a weighed filter, wash with cold alcohol, dry at 100° and weigh.

The remainder of the powder is boiled with dilute hydrochloric acid and filtered through a weighed filter, the part insoluble in the acid is collected on the same filter, washed with water, dried at 100° and weighed. This gives the amount of bile-pigments.

The acid solution is carefully evaporated to dryness and the residue weighed. This gives the total amount of inorganic matter. The ash is then redissolved in water, slightly acidified with hydrochloric acid. From this solution, the copper is precipitated as a sulphide and separated by filtration. In the filtrate, the magnesium, calcium and iron may be estimated quantitatively according to the usual methods. The copper sulphide is redissolved in dilute nitric acid, the excess of acid driven off and the solution diluted with water. From this solution the copper is precipitated by potassium hydrate and estimated as cupric oxide.

### PANCREATIC JUICE.

§ 40. Pancreatic juice, as obtained by means of a temporary fistula, varies in its composition as the animal feeds or fasts. During fasting, the secretion may entirely disappear or a thin, watery fluid may be obtained in small quantity. About six hours after taking a large meal, the secretion is abundantly poured out. This is a clear, colorless, somewhat viscid fluid, which has a decidedly alkaline reaction and a specific gravity varying from 1006 to 1010.

This secretion possesses the following digestive properties: (1) It readily converts starch into ferment sugar; (2) it digests fibrin forming peptons, leucin, tyrosin, glutamic acid and asparagic acid; (3) it emulsifies fats and separates neutral fats into glycerin and their respective acids. Concentrated acids, strong alkalis and many metallic salts arrest the action of the pancreatic juice; but the gastric juice does not contain sufficient acid to materially influence the action of the pancreatic juice, and, moreover, in the intestines, the alkalinity of the bile aids in destroying the acidity of the foods.

If pancreatic juice be kept for some time at the freezing point, the fluid forms a jelly-like mass, which, on being gently warmed, regains its original fluidity. Heated to 80°, pancreatic juice coagulates.

If this secretion be treated with a stream of chlorine gas, a white precipitate appears and after standing in a warm room

becomes rose colored. Alcohol throws down a precipitate which is soluble in water.

The pancreatic juice varies greatly in its composition and properties in different animals. The secretion of this gland in the sheep is thick and viscid; while that of the cat is a thin, watery fluid, is not ropy and contains but little pancreatin. In many fish the pancreatic secretion is acid in reaction, does not possess the power of converting starch into sugar and serves only to aid the gastric secretion in its action upon albuminous food. A similar pancreatic juice is obtained from the cul-de-sac in which the alimentary canal of some insects terminates. The glycerin extract of the honey bee converts cane sugar and starch into ferment sugar. (Hofmann.)

The various digestive properties possessed by the pancreatic juice are due to special ferments which have never been obtained in a chemically pure state, and which are imperfectly separated from one another.

§ 41. *Separation of the Ferments.*—Heidenhain has discovered a method of partially separating the ferments of the pancreatic juice. His method is essentially as follows: The pancreas is removed from a dog which has been killed six or seven hours after a full meal. Wash the organ with water in order to remove the blood; rub it up with ground glass or sand in a mortar; place the pulp in a beaker; cover with water and keep on the water-bath at a temperature from  $20^{\circ}$  to  $30^{\circ}$ , never above the latter, for two hours; filter through linen and press the residue; to the filtrate, which may be either neutral, acid or alkaline in reaction, add an excess of calcined magnesia; allow to stand for fifteen minutes, then filter, first through linen and then through paper. (This filtrate changes starch into sugar and digests albumen, but is without action upon fats, since the ferment peculiar to the fats has been removed by the magnesia.) Pour the filtrate into a flask capable of holding three times the volume of the filtrate; pour on the solution, without stirring, a solution of thick collodion equal in amount to one-third of the fluid; close the flask and shake thoroughly for five minutes; pour the mixture into a wide beaker and stir constantly in order to hasten

the evaporation of the ether. A precipitate is deposited in granules; bring the precipitate on a linen filter; treat the filtrate with a solution of collodion as before and bring the second precipitate upon the same filter. The filtrate now contains the starch-ferment, while the albumen-ferment is with the precipitate on the linen. (Set the filtrate aside for further examination.) Wash the precipitate thoroughly with alcohol of from 60 to 70 per cent; dry the precipitate by pressing it between folds of blotting paper; suspend it in a mixture of ether and alcohol in order to remove the collodion; filter and wash the residue on the filter well with ether; then dissolve in water. This solution digests fibrin, but is without action upon starch or fats.

Concentrate the solution containing the starch-ferment in vacuo to one-sixth its volume; add a large excess of absolute alcohol and allow to stand for three days. Collect the precipitated ferment on a filter and wash it with absolute alcohol; treat it with a mixture of two parts of alcohol and one part of water: this dissolves the ferment from the albuminous substances which have continued with it so far. Filter the solution and evaporate it to dryness in vacuo; dissolve the residue in a small quantity of water. This solution acts energetically upon starch in either neutral, alkaline, or acid solutions; it also possesses slight digestive action upon fibrin, all of the albumen-ferment not having been removed. This aqueous solution also contains traces of leucin, tyrosin and inorganic salts, from which it may be freed by dialysis.

§ 42. *Pancreatin*.—This is the name proposed by Heidenhain for that ferment which enables the pancreatic juice to digest fibrin. This substance does not exist preformed in the pancreas; for if this organ be taken directly from the animal after death, cut into fine pieces and covered with glycerin, and allowed to stand for a month, only a trace of pancreatin is extracted. Heidenhain thinks that it is probable that in the pancreas the pancreatin is combined with some albuminous substances; this compound is inert and only after it has been decomposed by macerating the organ in warm water, or by the addition of an acid, can the active pancreatin be obtained artificially. This

inert compound he designates as *zymogen*. The amount of this substance contained in the pancreas varies inversely with the amount of pancreatin in the secretion. Consequently while the latter is most abundant during the sixth and seventh hours after a meal, the inert compound is stored up in greatest quantity in the organ from the eighteenth to the thirtieth hour after a meal. The secreting cell of the pancreas of the fasting animal consists of an outer and inner zone; the former is readily stained by carmine, while the latter is stained with difficulty, or not at all. The inner zone is granular, while the outer is homogeneous or striated. When the animal fasts the inner zone widens, and the wider this zone the more zymogen can be extracted. During digestion the inner zone grows smaller, while the outer increases in size. It seems that the zymogen is furnished by the inner zone, which, in turn, obtains the zymogen or the substance from which the zymogen is formed, from the outer zone; while the outer obtains the material from the blood. During digestion the zymogen is poured out from the inner zone, while the outer continues to receive material; consequently the explanation why the former decreases and the latter increases is evident.

*Extraction of Zymogen.*—Remove the pancreas from an animal which has been without food for thirty hours; cover with glycerin immediately; cut into fine pieces and allow to stand for three days. This glycerin extract is without action upon fibrin; but if it be diluted with warm water or with water acidulated with acetic acid, pancreatin is formed and will digest fibrin in an alkaline solution.

*Properties of Pancreatin.*—Pancreatin is a yellowish-white powder, soluble in water and glycerin, insoluble in alcohol. The aqueous solution is without action or acts very slowly upon fibrin; but upon the addition of a .1 per cent. solution of sodium carbonate, fibrin is digested rapidly. The activity is lessened and finally destroyed by the farther addition of an alkali. Thus pancreatin differs from pepsin inasmuch as the former requires an alkaline, while the latter demands an acid reaction for the manifestation of its activity. Pancreatin is most active when freely supplied with oxygen; whether this be due to more rapid con-

version of zymogen into pancreatin, or whether the action of the latter consists in oxidizing, are questions not positively settled. Kühne designates this substance by the name *trypsin*.

§ 43. *The Products of Pancreatic Digestion of Albuminous Substances*.—Besides peptons, leucin, tyrosin, glutamic acid and asparagic acid, there are other substances produced by the action of the pancreatic juice upon albuminous substances. Among these is a xanthin-like substances, an aromatic acid, and a substance which causes the mixture to become red or violet on the addition of chlorine or bromine water. There are probably other unknown products. All of these, except pepton, are decomposition products, and in this respect pancreatic differs from peptic digestion. The amounts of leucin and tyrosin formed are quite large: thus Kühne found that a quantity of fibrin corresponding to 382 grams of dry substance, after being digested for six hours at from 40° to 48° with six liters of water and a pancreas (weighing 55 grams or 15.2 grams dry) furnished 31.6 grams of leucin, 13.3 grams of tyrosin and 211.2 grams of pepton. The leucin and tyrosin thus formed in the intestines are carried to the liver where normally they are broken up into urea and uric acid.

§ 44. *Amylolytic Ferment*.—The partial separation of this ferment from pancreatin has been given. It seems to be identical with the ptyalin of the salivary glands. The action of this ferment is, however, much more intense than that of ptyalin. At from 37° to 40° it instantly converts starch into sugar. At 12°, it transforms glycogen into sugar. The product of pancreatic digestion of starch is not wholly or even in greater part grape sugar as is generally stated. Digest some starch paste for ten hours with an infusion of a pancreas at 15°, concentrate the fluid to a syrup and add much alcohol and ether, dextrin will be precipitated. Remove the dextrin by decantation or filtration and add more ether, when a sugar agreeing closely with maltose in power of reduction and effect upon polarized light will be precipitated; while in the supernatant fluid a trace of glucose may be found.

§ 45. *The Fat Ferment*.—This has not been obtained free



from the others. An active extract may be obtained when a perfectly fresh pancreas is covered with a mixture of nine parts of glycerin and one part of a one per cent. solution of sodium carbonate, the whole rubbed up with sand or powdered glass, and allowed to stand for four days. This extract emulsifies fats and divides neutral fats into glycerin and fatty acids. As soon as sufficient fatty acid has been freed to neutralize the sodium carbonate, the action is arrested, since it can continue only in an alkaline solution. Shake some of this extract with olive oil, place upon the water-bath at 40° and allow to remain for some time. The reaction, ascertained by placing a drop on a strip of blue litmus paper, will soon become acid.

### INTESTINAL JUICE.

§ 46. From the duodenum a ferment can be obtained by extraction with glycerin, which bears a close resemblance to, and probably is identical in composition with, the *succus pyloricus*. It is strongly alkaline, and on being acidified with hydrochloric acid, digests fibrin. Intestinal juice also possesses two other ferments, by means of which it converts starch and cane sugar into grape and invert sugars. According to the observations of Busch in case of intestinal fistula, human intestinal juice does not convert cane sugar into grape sugar; but does readily convert starch into sugar, and acts energetically upon albuminous substances. The glycerin extract of the intestine of the sheep is without action upon starch. The secretion of the large intestines is also alkaline, is tenacious, turbid, contains albumen and is, outside the body at least, without action upon starch, albumen or fat. It is true that the food after it enters the large intestine generally possesses an acid reaction; but this acidity is due to fermentative changes in the food and not to the secretion of this part of the alimentary canal. That fermentation does go on, is shown by the nature of the intestinal gases. These vary greatly in kind and amount with the nature of the food; the gas from carnivora is rich in nitrogen, while the intestinal gas obtained from herbivora contains as much as fifty per cent. of methane, CH<sub>4</sub>. It is probable that these fermentative changes may fit some parts of the food for absorption; this is most likely

to be the case in herbivora. Evidently, food has been partially absorbed when administered per rectum and that this food is fitted for absorption by fermentation, is probable. According to Nencki, microscopic examination of the contents of the dog's intestine shows that there are different species of bacteria peculiar to the several parts of the canal. Some of the known products of these putrefactive processes are ammonia, carbon dioxide, hydric sulphide, methane, hydrogen, butyric acid, valerianic acid, acetic acid, indol, skatol and phenol. The three last are excreted partly unchanged in the fæces and partly in other forms in the urine. The office of the nascent hydrogen in forming hydrobilirubin from bile coloring matters has already been mentioned.

*Preparation of Intestinal Juice.*—Remove the small intestines from the animal as soon as it dies. Ligate one extremity and force water into the open end until the intestine is filled; temporarily fasten the open extremity and agitate the contents by moving through the hand and gently pressing upon the intestine. Remove the temporary fastening and pour out the water. Repeat the process of filling, agitating and emptying until the wash-water is transparent or but slightly turbid. Lay open the canal from one extremity to the other; remove the muscular layer from the mucous membrane; cut the latter into fine pieces; rub these up with powdered glass; cover with water; allow to stand for three hours and filter, first through cloth and then through paper. This solution converts cane sugar into invert sugar.

#### THE FÆCES.

§ 47. The fæces consist of undigested parts of the food more or less changed by the various physical and chemical agents to which they have been subjected and mixed with the secretions and transudations. The proportion of the fæces to the food varies with the kind of the latter, the condition of the digestive fluids and the movements of the intestines. Normally, the fæces of man are equal in weight to about one-eighth of the food averaging about 130 grams for the twenty-four hours.

The color of the fæces varies with the food and with the action of the liver. After a meal consisting of flesh, the fæces

are dark-brown; while after an exclusive milk-diet, they are yellow. When the biliary secretion is arrested, the fæces are light colored and when this secretion is excessive, they may be dark or green. In many diseases, the color of the excrement is decidedly changed; thus in diarrhœa, the stools are light colored and, in obstruction of the bile duct, may be perfectly colorless. From the fæces, traces of unchanged bile-pigments may often be obtained. Hæmatin is frequently present and in carnivora is often from the flesh of the food and, in any animal, may be present from bleeding of the walls of the alimentary canal. Only when the bleeding is in the lower part of the large intestine, is unchanged blood present in the stools.

The odor of the stools is due to indol, skatol, valerianic or butyric acid or hydric sulphide. Normally the odor is due to indol, ( $C_8H_7N$ ) and skatol ( $C_9H_9N$ ). Indol is obtained artificially as a final product in the reduction of indigo; it is a weak base, forms large colorless crystalline plates, which are freely soluble in hot, sparingly, in cold water. Indol is often formed during pancreatic digestion, but is supposed to be due to the presence of bacteria, since its formation is arrested if the pancreatic digestion be carried on in the presence of salicylic acid. Indol is recognized by its odor and by its reaction with nitrous acid. With this acid even very dilute solutions of indol produce a red color. This substance will be described more fully when discussing the coloring matters of the urine.

Normally, the reaction of the fæces is neutral or alkaline; but in certain diseased conditions, it is acid. The amount of solids varies from 174 to 317 parts in a thousand. The solids consist of earthy salts, skatol, excretin, taurin, cholalic acid, dyslysin, fats, sometimes acetic, lactic, butyric, and valerianic acids; while urea, hæmatin, hæmaglobin, albumen, bile-acids and bile-pigments are occasionally found.

*Skatol.* To five or six kilograms of fæces add eight liters of water, and 200 c. c. of acetic acid, and distil the mixture. Neutralize the distillate with sodium carbonate, then shake with ether and remove the ethereal layer. Evaporate the ether slowly, when an oily residue will remain. On standing this

forms a more or less colored crystalline mass which should be dissolved in hot water and allowed to cool, when skatol will form in snowy white crystals. Skatol may also be prepared by pancreatic putrefaction, as follows: Cover two kilograms of pancreas and one-half kilogram of muscle with eight liters of water, and allow to stand for five months. Then add acetic acid and distil the mixture. To the distillate add picric acid when the picrate of skatol will be precipitated, and will form in beautiful red needles. According to Nencki, 0.3 grams of pure skatol is furnished by this formula.

Skatol is distinguished from indol by the fact that the former is not colored red by fuming nitric acid. It is not present in the fæces of dogs nor in typhus stools.

*Excretin*.—This substance, which was discovered by Marcet, exists in human fæces, but not in those of the dog. It is non-nitrogenous, is represented by the formula,  $C_{78}H_{156}O_2S$ , and is prepared as follows: Extract the fresh fæces with hot alcohol and filter; to the concentrated filtrate, add milk of lime; collect and dry the precipitate which forms; extract the dried precipitate with a mixture of equal volumes of ether and alcohol; set the extract aside in a cool place. After six or eight days usually, excretin forms in fine, needle-shaped crystals; it can be purified by dissolving in boiling alcohol, from which it recrystallizes on cooling. Excretin melts at  $92^\circ$ , is insoluble in water, soluble in hot alcohol and ether.

Flint's stercorine is impure cholesterin (Hofmann.)

*Analysis of Normal Fæces*.—Hoppe-Seyler recommends the following process for the examination of normal fæces: First extract the mass with hot alcohol and filter. This filtrate contains fatty acids free or combined with alkalis, bile-acids, bile-pigments, traces of cholesterin and some inorganic salts. The fat may be detected by allowing some of the alcoholic solution to evaporate to dryness, adding a little water to the residue and examining under the microscope. The bile-acids and the bile-pigments may be recognized by evaporating the alcohol, dissolving the residue in water and applying Pettenkoffer's and Gmelin's tests. The cholesterin may be recognized by its crystalline form.

The mass insoluble in alcohol is now extracted with ether. The ethereal solution contains fats. The remaining residue is extracted with ether, acidified with hydrochloric acid; this dissolves out the palmitic and stearic acids which were combined with lime.

A quantitative estimation of the fats of the fæces is often desirable for diagnostic as well as experimental purposes. This is best accomplished in the following manner: Extract a weighed portion of the excrement first with a mixture of alcohol and ether and then with ether acidified with hydrochloric acid. Finally, wash well with ether. Unite these filtered extracts, add sodium carbonate and evaporate the mixture to dryness on the water-bath; wash the residue with water into a flask, add ether and shake thoroughly; allow to stand and draw off the ethereal layer with a pipette; shake again with ether and repeat this process as long as the ether takes up anything; this is known by allowing a few drops of the ethereal solution to evaporate and observing whether any residue remains or not. The united ethereal solutions are poured into a weighed beaker, evaporated to dryness, and dried at the temperature of the water-bath and weighed. If the fats which have combined with the sodium are to be estimated, acidify the aqueous solution with a few drops of hydrochloric acid, shake with ether, evaporate the ethereal solution, dry and weigh as before.

*Urea*.—This substance is often abundant in cholera-stools and is obtained as follows: Extract the fæces with cold water; filter; evaporate the filtrate to dryness on the water-bath; extract this residue with absolute alcohol; filter; again evaporate to dryness on the water-bath; dissolve the residue in a little water and add an equal volume of nitric acid. After some minutes, nitrate of uræa forms in rhombic tablets.

*Albumen*.—This is frequently present in the stools of diarrhoea. Filter the liquid fæces and test the filtrate for albumen with heat and nitric acid.

*Hæmatin*.—Extract the fæces with cold alcohol; boil the insoluble part with alcohol to which a few drops of sulphuric acid have been added, and filter. Concentrate this filtrate and

examine through the spectroscope (see hæmatin). Whether this evidence be positive or negative, proceed as follows: Saturate the solution with sodium hydrate and filter; evaporate the filtrate to dryness at a gentle heat; wash the residue with dilute nitric acid. Pure hæmatin now remains and may be recognized by its spectroscopic appearance and by the detection of iron in the ash. (Hoppe-Seyler).

*Meconium*.—The excrement which is passed by the newborn infant and which has accumulated during intra-uterine life, is of a dark-green or black color and consists of intestinal mucus and epithelium, with bile-acids and pigments and traces of cholesterin. The bile-acids and cholesterin may be extracted with boiling alcohol, from which the latter crystallizes on cooling; while the bile-acids may be obtained by evaporating the alcohol, dissolving the residue in a little water and applying Pettenkoffer's test. This excrement has an acid reaction, is odorless and exhibits, under the microscope, cylindrical epithelium, fat globules, and plates of cholesterin.

§ 48. *Intestinal Calculi*.—These are frequently found in herbivorous animals, seldom in man. They are especially likely to occur in horses which have been fed upon bran. These calculi consist principally of magnesian and ammonia-magnesian phosphates, consequently are soluble in acetic acid and reprecipitated from this solution on the addition of ammonium hydrate. When burned the odor of ammonia is given off. In horses, intestinal calculi have been found which weighed as much as fifteen pounds. Sometimes the very large calculi found in oxen and horses are light, of a grayish color and consists of grass and parts of plants held together by earthy phosphates.

Intestinal calculi have been found in persons who have lived principally upon oatmeal and coarse breadstuffs. These are seldom as large as a hazelnut and consist of phosphates fat and bran.

## BLOOD.

### HÆMOGLOBIN.

§ 49. Synonyms: Simon's *Hæmatoglobulin*; Lehmann's *Hæmatocrystallin*; Stoke's *Scarlet Cruorin*; Berlin's *Chromatin*;

also the *Oxyhæmoglobin* and *Erythrocrucorin* of various authors.

Hæmoglobin is the principal constituent of the red corpuscles of the blood of vertebrate animals. In man, the dog, pig, ox and many other animals, the red corpuscles are almost pure hæmoglobin, only traces of other substances being present; while in birds this coloring matter is associated with an albuminous substance. Healthy human blood contains, on an average, twelve per cent. of hæmoglobin; but it must be remembered that the amount varies at different times of the day and with other circumstances influencing the normal periodic changes of the individual. Arterial blood contains a somewhat larger amount than venous blood. In a person suffering with cholera, the blood, on account of its concentration, contains a much larger per cent. of hæmoglobin than is normal; while in leucocythæmia the per cent. is decreased. (Hofmann). Hæmoglobin is more abundant in carnivora than in herbivora, in the adult than in the young, and in the fasting than in the recently-fed animal.

The following table, taken from Hofmann's *Zoochemie*, shows the per cent. of this coloring matter in some of the domestic animals: One hundred grams of blood contain—

In the rabbit—	8.4 grams of hæmoglobin.
In the sheep—	11.2 grams of hæmoglobin.
In the ox —	12.3 grams of hæmoglobin.
In the pig —	13.2 grams of hæmoglobin.
In the hog —	13.8 grams of hæmoglobin.
In the cock —	8.5 grams of hæmoglobin.
In the duck —	8.1 grams of hæmoglobin.

Hæmoglobin exists not only in the blood corpuscle, but also in some muscles and in solution in the blood of some invertebrates, as, for instance, in the angle-worm. Amorphous hæmoglobin can be separated from the blood of man, crystals of hæmoglobin are obtained with difficulty; while the blood of the dog, cat, rat, goose and many other animals, readily yields the crystalline form.

§ 50. *Preparation*.—Stir fresh blood for ten or fifteen minutes with a piece of whalebone or a bundle of glass rods, and

filter through calico or linen, which has been freed from starch by having been previously washed and dried. To the filtrate, add ten times its volume of a mixture of one volume of a saturated solution of sodium chloride and nine volumes of water; place the beaker containing this mixture in a cool place, at or below  $0^{\circ}$ , and allow to stand for two days. During this time, the greater part of the blood corpuscles will have fallen to the bottom. Decant the supernatant fluid; stir up the corpuscles with a dilute solution of sodium chloride; allow them to subside and again decant the supernatant fluid; repeat this operation two or three times. By means of a little water, transfer the corpuscles thus freed from serum to a small beaker; add a large excess of ether; shake well; allow to stand and remove the ethereal layer which contains lecithin and cholesterin. Filter the red aqueous solution, from which the ether has been removed, through a fast filter; cool the filtrate to  $0^{\circ}$ , then add one-fourth its volume of alcohol which has also been cooled to  $0^{\circ}$ ; allow this mixture to stand at a temperature of from  $-5^{\circ}$  to  $-10^{\circ}$  for a few days, when hæmoglobin will be deposited in either the crystalline or amorphous form. If the blood used were that of the dog, rat, squirrel, or hedgehog, crystals will form so fast on shaking the corpuscles with ether, that the greater part of the hæmoglobin will rest in the crystalline form on the filter in the subsequent filtration. These crystals may be used for microscopic examination and then dissolved by digesting with a little water at  $35^{\circ}$  on the water-bath. Filter this solution; cool the filtrate to  $0^{\circ}$ ; add one-fourth its volume of alcohol, previously cooled to  $0^{\circ}$ , and allow to stand as recommended above. In this way, crystals of pure hæmoglobin are obtained. (Hoppe-Seyler).

It is sometimes desirable to obtain crystals of hæmoglobin from coagulated blood. Place a piece of the coagulum in a small beaker or test tube and set in a cold place, below  $0^{\circ}$ , and allow to stand for three days. To the blood add a few drops of water. Place a drop of the mixture on a glass slide, cover with a thin glass and leave for some hours in a cold place, when crystals will form and may be detected by the microscope.

To some blood of the guinea-pig, add half its volume of



water, shake well and allow to stand in a cool place, when a crystalline sediment forms. If a larger proportion of water is added, the crystals will not appear, or will be decomposed and replaced by an amorphous deposit.

To 5 c. c. of difibrinated blood add, drop by drop, water until a clear solution is obtained. To this add one-fourth its volume of alcohol and place the mixture in a platinum dish in a freezing mixture, when hæmoglobin crystallizes and may be recognized by microscopic examination. (Hofmann).

§ 51. *Properties.*—The crystals of hæmoglobin are mostly rhombic prisms, but vary in form and composition with the species of animal from which the blood is taken. Blood from the dog yields hæmoglobin, which forms in four-sided prisms; from the squirrel, in six-sided plates; from the goose, in rhombic tablets; from turkeys, in cubes and octohedrons; from the horse and man (when obtained in the crystalline form), in rhombic tablets and prisms.

The crystals of hæmoglobin vary also in the per cent. of water of crystallization which they contain and in the relative amount of each element represented in the molecule. The following table, taken from Hoppe-Seyler's *Handbuch*, S. 252, shows these variations:

	Per cent. of						
	Water.	C.	H.	N.	O.	S.	Fe.P <sub>2</sub> O <sub>5</sub> .
Crystals from the dog.....	3-4	53.85	7.32	16.17	21.84	0.39	0.43 —
Crystals from the goose.....	7	54.26	7.10	16.21	20.69	0.54	0.43 0.77
Crystals from the guinea-pig..	6	54.12	7.36	16.78	20.68	0.58	0.48 —
Crystals from the squirrel.....	9.4	54.09	7.39	16.09	21.44	0.40	0.59 —

It will be seen from this table that of the animals there represented, the dog furnishes hæmoglobin poorest, and the squirrel, richest in water of crystallization.

Again, the crystals vary in the degree of solubility in water; those obtained from the blood of birds are most sparingly, while those from the dog are most freely soluble in this menstruum. In direct proportion to their solubility, the crystals vary in the readiness with which they absorb water from the atmosphere; thus hæmoglobin from the blood of the raven is very sparingly soluble in water and is not at all hygroscopic. (Hofmann).

If the crystals be dried at  $0^{\circ}$  in vacuo over sulphuric acid, a brick-dust deposit remains. If the temperature rises above the freezing point during the process of drying, the hæmoglobin partially decomposes and a black residue remains.

The crystals of hæmoglobin, as well as an aqueous solution of the same, have the bright red color of arterial blood. The aqueous solution gives a feebly acid reaction, and is decomposed, with the formation of an albuminous substance which coagulates, on being heated to  $65^{\circ}$ .

The crystals or the aqueous solution of hæmoglobin contain oxygen, which is loosely held in combination and which may be removed by means of the air-pump or by various reducing agents. This oxygen is not recokoned in the ultimate analysis of this coloring matter, which has already been given. The term, *Oxyhæmoglobin*, is often used to designate this substance as it holds the oxygen, and in contradistinction to the hæmoglobin from which this oxygen has been removed. After the removal of the oxygen, the coloring matter dissolves more readily in water, but does not recrystallize or does so with great difficulty. The amount of this loosely combined oxygen which may be freed is constant; thus, measured at a pressure of one meter, the oxygen given off from one gram of pure crystals occupies 1.34 c. c.

If a dilute solution of oxyhæmoglobin be examined with the spectroscope, a very characteristic spectrum will be observed. A small portion of the red, and a larger portion of the blue end will be absorbed, while between the solar lines D and E will appear two bands. Of these, the one nearer D is the smaller, darker and more sharply defined; the other lies close to the line E and is less intense. These two band appear in very dilute solutions, being plainly visible in a thickness of 1 cm. of a solution of 1 gram of dry oxyhæmoglobin in 10,000 c. c. of water. On further dilution these bands finally disappear; the one nearer E being the first to become invisible. If, on the other hand, more concentrated solutions be used, the spectrum varies with the degree of concentration. With a .5 per cent. solution, the absorption of the blue end extends to the red-ward side of G; while of the two bands between D and E, the one nearer the

former covers that solar line. With an .8 per cent. solution, the two bands unite, and the only rays which pass through lie to the red-ward side of D.

If now an aqueous solution of oxyhæmoglobin be treated with a current of nitrogen or hydrogen gas, the brilliant hue of the solution is replaced by a purple color; the loosely combined oxygen has been removed and *reduced hæmoglobin* remains. The same effect is produced by adding to the solution of oxyhæmoglobin reducing agents, as the alkaline sulphides, ammonical solutions of tartrates (as tartaric acid added to a solution of ferrous sulphate, until a precipitate no longer occurs on the addition of sodium hydrate, and then the whole made alkaline with ammonium hydrate), finely divided tin or other metals.

Spectroscopic examination of a solution of reduced hæmoglobin reveals a spectrum entirely different from that of the oxyhæmoglobin: the two bands between D and E are replaced by a single broad band which is less distinct than either of the other two. Moreover, less of the blue end of the spectrum is absorbed; while the brightest part lies between B and C: thus, the red and blue rays pass through a solution of reduced hæmoglobin, consequently its color is purple.

If a solution of reduced hæmoglobin be shaken with air, oxygen is reabsorbed, oxyhæmoglobin is formed and will be recognized by the change in color from purple to scarlet and by the reappearance of the two bands in the spectrum. A concentrated solution of oxyhæmoglobin presents a dark spectrum with the exception of a red band between C and D; if a drop of a solution of sodium sulphide be added to the oxyhæmoglobin the light between C and D will be excluded and a bright band will appear between B and C. Now shake the solution with air and the spectrum of oxyhæmoglobin will reappear.

§ 52. *Physiology*.—By studying the chemical properties of hæmoglobin or the red corpuscle, a more exact knowledge of its physiology has been obtained than could have been secured in any other way. Vain conjectures, wild fancies, and strange theories have been proposed concerning the change of arterial into venous blood. For a long while, honest, earnest workers

have endeavored to ascertain the chemical properties of the red corpuscle. Brande, Gmelin, Lehmann and others worked diligently in this field and with partial success; and finally, Hoppe-Seyler succeeded in preparing hæmoglobin in its pure state. It is true that every particular concerning the red corpuscle is not yet understood and it may be that future years of investigation will yield more than the present possesses; but it becomes us to appreciate as fully as possible the benefits arising from a clear understanding of these facts. The light arising from the discovery of the chemical properties of hæmoglobin not only illuminates many hitherto dark corners of physiological science, but extends in all directions through the various departments of pathology.

Arterial blood contains much oxyhæmoglobin and but little reduced hæmoglobin; while venous blood is poor in the former and rich in the latter. It is true that if diluted venous blood be examined with the spectroscope the two bands which have already been described as characteristic of oxyhæmoglobin will appear: this is due to the fact that these bands are much more sharply defined than the one of reduced hæmoglobin. Consequently, in a mixture of these two substances, the oxyhæmoglobin, though it may be present in very small proportion, will be recognized on spectroscopic examination. If all of the oxyhæmoglobin should disappear from the blood, death would follow: this happens in the last stages of asphyxia and then the two characteristic bands cannot be obtained. As the blood leaves the left ventricle for all parts of the body, it contains much loosely combined oxygen; nearly all of the hæmoglobin exists in the oxidized condition. During its passage through the arteries and capillaries, the blood performs its great function, that of an oxygen carrier; recently received material from the alimentary canal must undergo certain chemical changes which are essential to the maintenance of animal heat, to the exercise of muscular activity, to the repair of various tissues, and to the production of thought. Waste material must be removed, solid tissue must be dissolved or converted into gases, organic matter must be changed into inorganic, poisons introduced from without and poisons generated within must be rendered inert

and fitted for excretion. The greater part of these changes are produced by the chemical activity of the loosely combined oxygen of the hæmoglobin and during its passage through the capillaries, this substance is deprived of a part of the oxygen and as reduced hæmoglobin is returned through the veins to the heart and lungs. As the venous blood passes through the lungs, the greater tension of the oxygen contained in the air cells over that of the blood causes the passage of the former into the capillaries and the reduced hæmoglobin is again oxidized and sent forth on its mission.

It has been stated that these facts illustrate some pathological conditions; for instance, in a case of phthisis an insufficient amount of oxygen is absorbed, oxyhæmoglobin is deficient and consequently many of the normal transformations of the body are completely or partially arrested. In such a case, large quantities of oxalate of lime will be found in the urine; the carbon of the food and of the waste material from the tissues is only partially oxidized and that which should have been exhaled from the lungs as carbonic acid, is excreted by the kidneys as oxalic acid. Again, in the condition of venous stasis arising from feeble action of the heart, the blood stagnates in the veins, becomes loaded with poisons, is not carried to the lungs with due rapidity and those nitrogenous parts of food and tissue, which normally are converted into and excreted as urea, appear in the urine as uric acid free or combined.

§ 53. *Compounds.*—Besides oxygen hæmoglobin takes up some other substances in a similar manner. It must be remembered that the association or dissociation of oxygen does not affect the molecular arrangement of the hæmoglobin itself. It is true that this combination is a chemical one, but the oxygen is held so loosely that it is replaced without injury to the structure of the hæmoglobin molecule; thus the red corpuscle receives its oxygen in the lungs and loses the same in the systemic capillaries and is not itself materially changed. Carbon monoxide (C O) has the power of freeing oxygen from oxyhæmoglobin and of forming carbon monoxide-hæmoglobin.

Treat a warm concentrated aqueous solution of oxyhæmo-

globin for a short time with a current of carbon monoxide ( $\text{CO}$ ); the oxygen will be liberated and an equal volume of the other gas will be taken up. Cool the solution to  $0^{\circ}$ ; add one-fourth its volume of cold alcohol; allow to stand for twenty-four hours exposed to a temperature at or below the freezing point, when beautiful, purple colored, four-sided prisms of this compound will appear. These crystals are more permanent and less freely soluble in water than those of oxyhæmoglobin. The spectrum of this compound is very similar to that of hæmoglobin, however the two lines are less distinct and regular and on accurate measurement will be found a little farther toward the violet end; also more blue light passes through. This spectrum is not so readily destroyed as that of oxyhæmoglobin, the addition of ammonium sulphide causing the disappearance of the bands only after several hours. Carbon monoxide-hæmoglobin is decomposed by arsenietted hydrogen gas.

The combination of carbon monoxide with hæmoglobin is stronger than that of oxygen with the same; thus, while oxygen is readily removed from oxyhæmoglobin by a current of carbonic oxide, the latter is but slowly freed from its compound by being treated with oxygen gas. Continued agitation with oxygen converts carbon monoxide-hæmoglobin into oxyhæmoglobin; probably the carbon monoxide ( $\text{CO}$ ) is first changed into carbon dioxide ( $\text{CO}_2$ ).

A study of the properties of carbon monoxide hæmoglobin explains the poisonous effects of inhaled carbon monoxide. When this gas is taken into the lungs, it combines with the reduced hæmoglobin, gives the blood a bright cherry-red color, and destroys its function as an oxygen carrier. Moreover, since this gas is but slowly displaced by oxygen, the animal dies of suffocation. The blood of an animal poisoned with this gas will often hold its color for days or weeks. It also manifests a different reaction with sodium hydrate from that of normal blood. If the latter be defibrinated and treated with twice its volume of a solution of sodium hydrate (specific gravity 1.3), a brown gelatinous mass separates and when this is spread upon a clean porcelain surface, it presents a dirty, greenish-brown tint.

If blood from an animal poisoned with carbon monoxide be treated in the same way, a cherry-red coagulum (which if spread upon a porcelain surface will present a red, slowly changing into a dark-brown color, appears.

Render a concentrated aqueous solution of oxyhæmoglobin feebly alkaline with barium hydrate and treat it with pure nitrous dioxide gas ( $\text{N O}_2$ ). This compound is more permanent than the corresponding one with carbonic oxide. Blood containing nitrous dioxide-hæmoglobin is of a bright red color and without the purple tint of the carbonic oxide compound. Spectroscopic examination reveals two bands, identical in position but different in appearance from those of oxyhæmoglobin. The bands of the nitrous dioxide compound are at first very faint, gradually growing darker, but never becoming so dark and distinct as those of oxyhæmoglobin.

An insoluble form of hæmoglobin is sometimes found in cysts. It appears as a brick-red deposit, consisting of corpuscles which are insoluble in water and alcohol, permanent at ordinary temperature; but are decomposed by acids or alkalis in the same way as hæmoglobin is decomposed. The ash of these corpuscles contains as much iron as that of hæmoglobin; besides iron, carbonate of lime is found in the ash. (Hoppe-Seyler).

§ 54. *Detection of Hæmoglobin.*—Since this coloring matter differs from most others of the animal world in not being precipitated by basic acetate of lead, nor by this reagent in the presence of ammonia, it is easily separated from any mixture. In making this separation, the basic acetate of lead should be added as long as the precipitate increases; but an excess should be avoided, because methæmoglobin and other substances which may be present are soluble in an excess of the basic acetate solution, and moreover, such an excess may cause the decomposition of the hæmoglobin. This separation should be made with the substance under examination and the reagents subjected to a temperature of, or as near  $0^\circ$  as possible. After other coloring matters and impurities have been removed by precipitation with the basic acetate of lead, and filtration, the filtrate is

tested for the presence or absence of hæmoglobin with the spectroscope. As a confirmatory test, the change of color and spectroscopic appearance on the successive additions of reducing agents and oxygen may be observed. Finally, if a portion of the solution be evaporated on the water-bath at 40° to 45° and to the residue on a watch crystal, a drop of a dilute solution of sodium chloride and a few drops of glacial acetic acid be added, and the acid be evaporated to dryness on the water-bath, crystals of hæmin will form and may be recognized on microscopic examination.

§ 55. *Decomposition.*—The products of the decomposition of hæmoglobin have not been satisfactorily studied. Evidently it can be broken up with the formation of at least one other coloring matter and one or more albuminous substances. If an aqueous solution of oxyhæmoglobin be allowed to stand for some hours at ordinary temperature, and then be examined with the spectroscope, an absorption band will appear between C and D, nearer the former, and it will also be found that a brownish precipitate will be formed on the addition of a few drops of a solution of basic acetate of lead. Moreover, a brownish substance, soluble in water and giving the above spectroscopic appearance and reaction with the lead solution, is not unfrequently found in cysts of various kinds into which blood had previously extravasated. This substance has been named *methæmoglobin* by Hoppe-Seyler, who recommends the following method for its detection: If the absorption band between C and D is not sufficiently distinct, add to the solution basic acetate of lead as long as a precipitate forms; collect this precipitate upon a filter; suspend it in water; add to the mixture sodium carbonate until the coloring matter is completely dissolved and the lead precipitated as a carbonate; filter, and examine the filtrate with a spectroscope. Besides the spectroscopic appearance, the formation of hæmin crystals, according to the method already given, and the detection of iron in the ash may be employed as confirmatory tests.

Methæmoglobin contains an albuminous substance which on further decomposition is set free and *hæmatin*, a non-albuminous coloring matter, remains.



§ 56. *Quantitative Estimation of Hæmoglobin.*—The quantity of hæmoglobin contained in blood may be estimated either by ascertaining the amount of iron present, by comparing the intensity of the color with that of an aqueous solution of a known quantity of hæmoglobin, or by ascertaining by means of the spectroscope the degree of dilution necessary to allow the transmission of the red rays only.

(1) By estimating the amount of iron in a weighed or measured quantity of blood, the amount of hæmoglobin may be calculated. In this method it is assumed that all of the iron obtained from the ash came from the hæmoglobin. It is known that hæmoglobin contains .42 per cent. of iron, which is equivalent to .60 per cent. of ferric oxide ( $\text{Fe}_2\text{O}_3$ ); consequently, if the amount of iron be ascertained and its equivalent of ferric oxide be calculated, and the amount of the latter be multiplied by 166.7, the result will represent the quantity of hæmoglobin. A weighed or measured quantity of the blood is evaporated to dryness; the residue is deprived of all organic matter by heat; the ash is dissolved in pure dilute hydrochloric acid; the solution filtered and boiled with small pieces of pure zinc until it becomes colorless, or all of the iron is reduced to the condition of a ferrous compound. In the whole or a measured portion of this solution, the amount of iron is estimated volumetrically with a standard solution of potassium permanganate.

The standard solution of potassium permanganate should be graduated with the greatest care. For this purpose weigh out .7 gram of pure double sulphate of iron and ammonia. This salt contains one-seventh of its weight of iron, consequently .7 gram contains .1 gram of metallic iron. Dissolve this weighed portion of the salt in water acidified with hydrochloric acid and dilute the solution to 50 c. c. To this solution in a beaker, add from a burette, a solution of potassium permanganate of indefinite strength drop by drop (constantly stirring the mixture) until a pale rose color appears and remains on stirring. Note the number of c. c. of the permanganate required and which represents .1 gram of iron. From this, the value of each c. c. of the permanganate solution is calculated and marked upon the bottle.

Suppose that 20 c. c. of the permanganate solution were required to produce the rose color, then 20 c. c. are equivalent to .1 gram of iron and 1 c. c. will represent .005 gram.

The whole or a measured portion of the solution of blood-ash is now diluted to 50 c. c., and to this in a beaker, the permanganate solution is added as above until the pale rose-color remains; the number of c. c. of the standard solution used are noted, and from this, the amounts of iron, of ferric oxide and of hæmoglobin are calculated from the relations between these substances as already given.

(2) Hoppe-Seyler estimates the amount of hæmoglobin by comparing the intensity of the color with that of a normal solution of the pure crystals, according to the following method which is taken from the Handbuch: Crystals of hæmoglobin are prepared from the blood of the dog, goose, or guinea-pig, preferably from the latter, purified as already directed (p. 72) and dissolved in water at 0° and filtered. Exactly 50 c. c. of this solution are poured into a porcelain crucible (the weight of which is known) evaporated to dryness on the water-bath, dried at 110°, cooled over sulphuric acid and weighed. From this the amount of hæmoglobin in each c. c. of the solution of crystals is estimated. From this solution (which is to be kept in a clean, corked flask) 10 c. c. are taken and diluted with from 10 c. c. to 60 c. c. of water and this is known as the dilute normal solution.

Dilute a small weighed quantity (not exceeding 20 grams) of the blood to be examined, previously defibrinated, to 400 c. c. by the addition of distilled water. For the comparison of the color, two similar cylindrical flasks may be used; but it is better to have two vessels, each of which is made of two parallel plates of glass, which are 1 centimeter apart, and whose edges on three sides are united by metallic strips, thus forming a deep, thin vessel, the bottom and two sides of which are 1 centimeter broad and made of metallic strips, while the remaining two sides are formed by the plates of glass. Such an instrument is made and known as a hæmatinometer. Fill one of the glass cases or cylinders with the dilute normal solution; into the other pour 10 c. c. of the dilute blood solution. Both vessels are placed side by

side on white paper, and so that the light will pass through. The blood solution will always be darker than the normal. To the former add water, a c. c. at a time, stirring, until the color of the two solutions is the same. Note the amount of water which has been added to the 10 c. c. of dilute blood. It is necessary to make one or more confirmatory tests, using different dilutions of the normal solution. In order to do this, pour the contents from each of the vessels and cleanse the same thoroughly. Into one, pour 20 c. c. of the dilute normal solution and add 10 c. c. of water; into the other, pour 10 c. c. of the dilute blood solution and add water as before until the color of the two is the same. Then dilute the normal solution by the farther addition of 30 c. c. of water, and add water to the blood until the color is again the same. By these repeated experiments the chances of error are diminished. When the color of the two solutions is the same, equal volumes of the two solutions contain the same amount of hæmoglobin, and from this the per cent. of this coloring matter in the blood may be calculated.

To illustrate this, suppose that 100 c. c. of the dilute normal solution contain .12 grams of hæmoglobin, and that it required the addition of 30 c. c. of water to reduce 10 c. c. of the dilute blood solution to the same color as that of the dilute normal solution; then each 10 c. c. of the 400 c. c. would require dilution to 40 c. c.; or for every 10 c. c., 30 c. c. of water must be added, or 1200 c. c. of water must be added to the 400 c. c.; consequently, 1600 c. c., the amount which would be if the whole of the blood were reduced to the color of the dilute normal solution, contain 1.92 grams of hæmoglobin. Suppose that the quantity of blood weighed for this estimation was 15 grams, then in this case 15 grams of blood would contain 1.92 grams of hæmoglobin, which is 12.8 per cent.

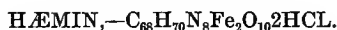
This method is easy of application and the normal solution may be kept for a week without decomposition; but the formation of the crystals is accomplished with facility only in cold weather.

(3) The third method of estimating the per cent. of hæmoglobin contained in blood is known as that of *Preyer*, and is as

follows: Place a concentrated aqueous solution of hæmoglobin crystals in a hæmatinometer in front of the slit of a spectro-scope; the light used being that of a petroleum lamp. To this, distilled water is added, with constant stirring, from a pipette graduated to one-hundredth of a c. c. as long as only red rays are transmitted, or until the green begins to appear. The per cent. of hæmoglobin in this solution is estimated by evaporating a measured portion to dryness, drying and weighing as given in the preceding method. In this way the per cent. of hæmoglobin required to allow the transmission of the red rays and the faint appearance of the green is ascertained.

The fresh blood, to be examined, is defibrinated by whipping, but is not filtered; a certain measured portion, (perhaps .5 c. c.) is taken up with a pipette and placed in the hæmatinometer, care being taken that the position of the lamp is the same as when examining the solution of crystals. Water is now added from the finely divided pipette as before until the green begins to appear. If now we represent the per cent. of hæmoglobin required in the solution of crystals by  $k$ , the volume of the blood placed in the hæmatinometer by  $b$ , and the volume of water added to the blood by  $w$ ; then  $x$ , the per cent. of hæmoglobin in the blood will be found by the following equation:

$$x = \frac{k(w+b)}{b}.$$



§ 57. This substance, which is the chloride of hæmatin, does not exist preformed in the blood, but is prepared from hæmoglobin.

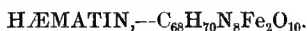
*Preparation.*—Sufficient crystals of hæmin (known also as Teichmann's crystals) for microscopical examination may be obtained as follows: A small quantity of dry blood is rubbed up with a few crystals of sodium chloride; the powder is placed on a glass slide; a fine thread or hair is laid through the powder across the slide so as to afford a means of escape to the bubbles of gas; a few drops of glacial acetic acid are added, and the whole is covered with a thin glass. The slide is now placed on

the water-bath and gently heated as long as air bubbles pass off. It is then removed, and the remaining acid is allowed to evaporate spontaneously. On examining this slide with the microscope, reddish-brown crystal of hæmin with a metallic lustre will be observed. (If the crystals do not appear the residue should again be warmed with the acid, and it may be necessary to repeat the process several times before a satisfactory result is obtained). Other objects, as colorless crystal of sodium chloride and acetate with threads of coagulated albumen, will be seen; but the color of the hæmin crystals will render their identification easy.

Hæmin in quantity may be prepared as follows: The corpuscles of defibrinated blood are freed from serum according to the method given for the preparation of hæmoglobin. The pulp of the corpuscles is transferred to a flask with a little water and shaken with half its volume of ether. After standing for a while, the ether is removed and discarded; the aqueous solution of the coloring matter is filtered and allowed to stand in a shallow dish at 50° until it acquires a syrupy consistency. This is shaken with from 10 to 20 volumes of glacial acetic acid, and the mixture heated on the water-bath for two hours. By this time the coloring matter will be converted into hæmin crystals, and the albumen will be partially dissolved. The deposit is stirred up and the whole is transferred to a large beaker and three volumes of water are added. After two or three days, the supernatant liquid is decanted from the crystalline deposit; the latter is washed repeatedly with water by decantation, and then heated for several hours with glacial acetic acid, which dissolves remaining traces of albumen. The crystalline deposit is again washed with water by decantation, then collected upon a small filter and washed first with alcohol and then with ether.

The crystals of hæmin have a reddish-brown or bluish-black color, a metallic tint, and are odorless and tasteless. When rubbed up, they form a yellowish brown powder. The powder and crystals are permanent at ordinary temperature and exposure; however, if the air contains a great excess of

ammonia, hæmin is gradually decomposed on exposure, with the formation of ammonium chloride and ammoniacal hæmatin. The crystals form in rhombic plates with variations of many kinds; but those from the blood of various species of animals have no characteristic form, or the form of the crystals is not determined by the animal from which the blood came. Hæmin is insoluble in water and very sparingly soluble in hot alcohol or ether, soluble with decomposition in alkalis, forming an alkaline chloride and hæmatin. It may be heated to  $200^{\circ}$  without decomposition but when the temperature is raised above this point, the hæmin is destroyed, hydrocyanic acid being given off and ferric oxide remaining as a residue. Compounds similar to hæmin are formed by the action of hydriodic and hydrobromic acids upon hæmoglobin. The iodide of hæmatin is a little darker than the chloride and the former has a violet tint; while the bromide is of a lighter red color than either of the other two.



§ 58. Hæmatin is frequently found in old blood extravasations and in the intestines. In the former instance, it comes from the decomposition of hæmoglobin; in the latter from the action of the gastric juice upon the blood contained in the food, or it may have already existed as hæmatin in the food. For the reasons just given, hæmatin is frequently found in the fæces of the carnivora. It appears in the urine in certain diseased conditions of the kidney and in cases of arsenic poisoning.

*Preparation.*—Boil hæmin crystals with glacial acetic acid, then wash them well with water, then with alcohol and ether. Dissolve the crystals in pure dilute potassium hydrate and filter; to the filtrate add dilute sulphuric acid; collect the brown precipitate which forms and wash this with water until the filtrate no longer gives a test for chlorine on the addition of silver nitrate. The hæmatin now freed from chlorine is warmed at first gently and then heated to from  $120^{\circ}$  to  $150^{\circ}$  until dry.

Hæmatin is an amorphous, bluish-black substance, with a metallic luster and forms a dark-brown powder, insoluble in water, alcohol, ether and chloroform; soluble in dilute alkalis. It is soluble in acidified alcohol, but insoluble in slightly acidified water. Hæmatin may be heated to  $180^{\circ}$  without decomposition, but when the temperature is raised much above this point, it is decomposed, hydrocyanic acid being given off and pure ferric oxide remaining as a residue. The amount of ferric oxide which remains is 12.6 per cent. of the weight of the hæmatin.

An alkaline solution of hæmatin, when examined in thin layers by transmitted light, presents a beautiful red color; when the light passes through thicker layers of the same solution, an olive-green tint is observed. Acid solutions have a brown color which is not influenced by variations in the volume of fluid through which the light passes. Both solutions absorb the violet end most notedly and the extreme red end the least. A solution containing .015 gram of hæmatin, one centimeter thick, presents an illy-defined absorption band between C and D, covering the latter. Hæmatin dissolved in alcohol acidified with sulphuric acid and placed before the spectroscope gives a band near C, between that line and D; another, less sharply defined, much broader and disappearing sooner on dilution, between D and F. This last band, by careful dilution, is broken into two bands of unequal distinctness; the one near F being the darker, the brightest interval being between E and b. A very much smaller band appears on dilution between D and E, near D. After treatment with ammonium sulphide, zinc tartrate, or other reducing agents, the solution of hæmatin changes its color, and on spectroscopic examination presents a small, dark, sharply-defined band between D and E, nearer the former, and a paler band which covers the lines E and b, the band of hæmochromogen. (Hoppe-Seyler).

If potassium cyanide be added to an alkaline solution of hæmatin, the color immediately becomes reddish-brown, and on spectroscopic examination, a broad, illy-defined band sim-





chloroform, soluble in alkalis and in alcohol acidified with sulphuric acid.

If a solution of hæmoglobin be reduced by means of a current of hydrogen and then be decomposed by alcohol containing either potassium hydrate or sulphuric acid in vessels from which the air is excluded, a new coloring matter is formed. This substance dissolves in dilute sodium or potassium hydrate, forming a beautiful purple solution, which gives characteristic absorption bands on spectroscopic examination. This is Hoppe-Seyler's *Hæmochromogen* and is represented by the formula,  $C_{34}H_{36}N_5FeO_5$ . It readily takes up oxygen and is converted into hæmatin.

#### PLASMA.

§ 60. We have seen that the principle office of the red corpuscle is to serve as a vehicle for carrying oxygen to the various tissues of the body; but there must be some agent to convey the corpuscle, to bring to the tissues material for repair and to remove the debris. Oxygen alone can not support life; there must be something to combine with the oxygen in order to produce animal heat. Moreover, this combustion must go on in every part of the body; even if it be true that the solid tissues enter but little into those chemical changes whereby life is supported, it is still necessary that combustion should take place in every organ. Let us suppose that the blood as it leaves the heart contains all of the oxygen and all of the material to be consumed, still, life could not be maintained did this oxidation become complete immediately, or take place in one organ only; the muscles of the arm and of every other part of the body alike need the production of heat within themselves before they can contract and relax; the brain requires combustion within its substance, whether of its substance or not, before it can act. The plasma serves as the channel for the transmission of material which supports life and of that which is the product of decay. It is, as Bernard said, the internal medium which bears the same relation to the tissues as the external medium, the world, does to the individual. The composition of the plasma is necessarily

very variable: at one time it may be bearing that which strengthens the body and elevates the mind; at another time it may contain poisons which injure both body and mind.

In order to obtain a large quantity of plasma the following method may be used: Allow the blood from a vein of a horse to fall into a tall, narrow beaker which is surrounded by a freezing mixture. After two or three hours, three layers will be observed: the lowest one is colored red and consists of the red corpuscles; above this and occupying not more than one-twentieth the space, is a layer of white corpuscles, while the upper part of the cylinder contains the plasma, which may be drawn off into another cooled cylinder.

Plasma kept at a temperature below  $0^{\circ}$  is a somewhat viscid, yellowish, strongly alkaline fluid. When the temperature is allowed to rise above  $0^{\circ}$ , the plasma is transformed into a jelly-like mass which gradually contracts and presses out a fluid resembling plasma in appearance and known as *serum*.

§ 61. *Coagulation*.—When blood is drawn from a vein and subjected to ordinary temperature, it is soon transformed into the jelly-like mass mentioned above. The coagulation of blood may be hastened or retarded by many agents; thus the higher the temperature, within certain limits, the more rapid the coagulation; while at or below  $0^{\circ}$  coagulation does not take place; and again by the addition of large quantities of some neutral salts, as magnesium sulphate, the formation of the clot may be prevented. In the latter instance, coagulation takes place after the addition of a sufficient quantity of water.

The less oxygen and the more carbonic acid blood contains, the more slowly will it coagulate; for this reason arterial blood coagulates more rapidly than venous. This process goes on more rapidly in blood which is poor in morphological elements than in that which is rich in the same constituents; for this reason the blood of a hydræmic person coagulates rapidly.

The causes of coagulation and the chemical changes which take place in the blood during the formation of the clot are

not fully understood; but the labors of Denis, Schmidt and others have been of great value and justify us in entertaining the belief that soon the mystery of this process will be removed by chemical investigation.

#### PLASMIN.

§ 62. *Preparation.*—Prevent the coagulation of plasma by cold and remove it from the corpuscles according to the method already given; or gently mix the blood as it flows from the vein with about one-third its volume of a saturated solution of magnesium sulphate, allow to stand until the corpuscles sink, and then remove the supernatant fluid. In either case, plasma free from corpuscles is obtained; by the first method coagulation is prevented by the cold, and would take place if the temperature were raised; by the second method coagulation is prevented by the presence of the neutral salt, and would take place if the solution were diluted. To plasma obtained by either of these methods, add sodium chloride to saturation, when a white precipitate will appear. Wash this precipitate with an aqueous saturated solution of sodium chloride, then dissolve it in a little water and filter through a fast filter. Allow the filtrate to stand exposed to an ordinary temperature, and soon it will be observed to coagulate just as the plasma did. It is evident from this that the coagulation of plasma is dependent upon this substance which has been precipitated by the sodium chloride, and which is called *plasmin* by its discoverer, Denis.

*Properties.*—Is this plasmin a compound body, and if so, what are its components? Serum nor hydrocele fluid either clots when kept separately; but if the two be mixed, coagulation occurs just as it does in plasma. Thus if some filtered hydrocele fluid be kept at from 38° to 40°, no coagulum appears, and the fluid will remain clear until decomposition takes place; but if a little serum be added, the mixture soon clots. This seems to be an answer to the question. Plasmin is a compound containing at least two substances, one of which is present in serum and the other in hydrocele fluid. By the labors of A. Schmidt each of these has been isolated,

and the one from serum is known as *fibrinoplastin*, *paraglobulin*, or *fibrinoplastic globulin*; while the one from hydrocele fluid is known as *fibrinogen*. Both of these are present in plasma and the plasmin of Denis is a mixture of fibrinoplastin and fibrinogen.

#### FIBRINOPLASTIN.

§ 63. *Preparation*.—Dilute serum from the blood of the horse or ox with ten times its volume of water, add a few drops of dilute acetic acid, not sufficient to destroy the alkalinity, and then treat with a current of carbonic acid gas. Wash the granular precipitate of fibrinoplastin, which falls, with water, until the wash-water no longer contains chlorides (tested for with argentic nitrate) or albumen (tested for with acetic acid and potassium ferrocyanide).

*Properties*.—Fibrinoplastin is insoluble in pure water, soluble in water containing much oxygen, soluble in dilute solutions of sodium chloride, sodium phosphate, and some other neutral salts. Dissolved in the above solutions, fibrinoplastin retains its active properties; for instance, if such a solution be added to hydrocele fluid, coagulation will take place. It is also soluble in acetic acid, but solution by this solvent destroys the activity of fibrinoplastin. The following table, taken from Hofmann's *Zoochemie*, shows the amounts of various substances required in 100 c. c. of water in order to dissolve one gram of paraglobulin:

0.002 grams of sodium hydrate.
0.017 grams of sodium carbonate.
0.034 grams of sodium bicarbonate.
0.092 grams of sodium phosphate.
1.974 grams of sodium chloride.
0.046 grams of acetic acid.

Besides the method already given for its preparation, fibrinoplastin may be obtained by saturating serum with sodium chloride, when it falls as a flaky precipitate. If this precipitate be collected and treated with distilled water, it dissolves; because, when prepared in this way, the precipitate holds sufficient sodium chloride to cause the distilled water to act as a dilute solution of that salt. It is thus seen that while a saturated

solution of common salt precipitates fibrinoplastin, a dilute solution of this substance dissolves this fibrin factor.

A filtered solution of fibrinoplastin in a dilute solution of sodium chloride does not clot; but if such a solution be added to hydrocele fluid, coagulation takes place. If the serum from which the fibrinoplastin has been removed be added to hydrocele fluid, no coagulum appears; thus it seems evident that one of the fibrin-factors is in excess in the plasma, and that this excess remains in the serum and may be extracted by the methods given.

#### FIBRINOGEN.

§ 64. *Preparation.*—To a specimen of hydrocele fluid, which has been found to coagulate serum, carefully add finely-pulverized sodium chloride to saturation. As soon as this point is reached, fibrinogen falls as a flaky precipitate and may be collected upon a filter, washed with a saturated solution of sodium chloride and dissolved in a little distilled water. This solution by itself does not clot; but when added to serum causes coagulation. If the hydrocele fluid, from which the fibrinogen has been removed, be added to serum, coagulation does not occur. Again if a solution of fibrinoplastin, obtained by saturation with sodium chloride from serum, be added to a solution of fibrinogen, obtained by the same method, from hydrocele fluid, coagulation takes place in a normal manner; while if the serum freed from its fibrinoplastin and the hydrocele fluid freed from its fibrinogen be mixed, no clot forms.

If instead of the saturation method, fibrinoplastin and fibrinogen be prepared by precipitation with carbonic acid, a mixture of the solutions of the two does not clot at all or does so imperfectly. This has given reason to suspect that either the fibrinoplastin or fibrinogen, as prepared by the saturation method, is a mixture of one or more substances. A. Schmidt has succeeded in isolating a third substance which he calls *fibrin-ferment*.

#### FIBRIN-FERMENT.

§ 65. *Preparation.*—To some serum add 20 times its volume of alcohol and allow the precipitate which forms to stand

under alcohol for about three months. Dry the hardened precipitate over sulphuric acid, pulverize it and extract with water. Treat the aqueous solution with a current of carbonic acid gas and filter; repeat this as long as the solution gives any reaction for albumen. If this solution freed from proteids be added to a mixture of fibrinoplastin and fibrinogen, prepared by the carbonic oxide method, coagulation occurs readily.

#### FIBRIN.

§ 66. The product of coagulation is fibrin. According to the views of Schmidt, it arises from the combination of fibrinoplastin and fibrinogen, in the presence of the ferment. Hammarsten thinks that fibrin is simply converted fibrinogen and that fibrinoplastin does not enter into the composition of fibrin, but acts as a ferment rendering the transformation of the fibrinogen possible.

*Preparation.*—Whip freshly drawn blood with a piece of whalebone or with a bundle of glass rods and collect the coagulated fibrin on a filter; or take the clot from blood which has coagulated spontaneously; cut the fibrin into small pieces; place these in a linen sack; press and rub under water, changing the water as soon as it becomes colored, until the color is no longer imparted to the fluid. Then place the sack containing the fibrin in a two per cent. solution of sodium chloride and allow to stand, with frequent agitation, for two days. In this way, traces of globulins are removed. Then place the fibrin in distilled water for 12 days, changing the water daily; cover the fibrin with alcohol and allow to harden; cut into fine pieces and extract with ether in order to remove any fat. Even when prepared in this way, fibrin is not perfectly pure, but contains traces of fat, white corpuscles and inorganic salts.

*Properties.*—Fibrin is insoluble in water, alcohol and ether, soluble in dilute alkalis forming albuminates. When digested with a two per cent. solution of HCl, fibrin is transformed into a semi-transparent, jelly-like mass. By the action of gastric juice, fibrin is converted into peptons, the change being a chemical one and not one of simple solution. If the gastric

juice contains but little pepsin the products of the digestion of fibrin with this fluid will be precipitated by neutralizing the solution. By the action of pancreatic juice, fibrin is transformed into peptons, tyrosin and leucin. Fibrin contains 52.6 per cent. of C, 17.4 of N, 21.8 of O, 7.0 of H, and 1.2 of S.

*Estimation.*—It is sometimes desirable to estimate the amount of fibrin in a specimen of blood. This is best done by the following method which is taken from Hoppe-Seyler's Handbuch: A small beaker with a rubber cap is needed; through a small opening in the center of the cap is passed a piece of whalebone shaped like an oar. This should be of such a length that when the cap is placed on the beaker, the tip of the wide end of the bone should just touch the bottom of the beaker, while the other end should project above the cap sufficiently to be grasped and moved easily with the hand. The apparatus is carefully cleansed, dried and weighed. The cap is removed and from 30 to 40 c. c. of the blood to be examined are placed in the beaker. (If plasma is to be examined, it is removed with a pipette from the beaker surrounded by the freezing mixture). The cap is now replaced and the blood stirred vigorously for 10 minutes with the bone. The apparatus with its contents is now weighed. The difference between the two weights now found will be the weight of the blood taken. After weighing, the beaker is filled with water, the contents well mixed and then allowed to stand. As soon as the fibrin has completely subsided, the supernatant fluid is decanted into another beaker. By means of a dilute solution of sodium chloride, the fibrin is transferred to a small weighed filter and washed with distilled water until the filtrate is colorless. With a small, clean pair of pincers, any pieces of fibrin which may be found clinging to the whalebone are removed and placed upon the filter. Finally, wash the fibrin several times with boiling alcohol in order to remove any fat. Then dry at from 110° to 120° in the air-bath, cool over sulphuric acid and weigh.

If it be desired, the decanted fluid and the wash-water,

may be mixed and the amount of hæmoglobin which they contain, estimated according to one of the methods given for the estimation of this coloring matter.

The addition of a little sodium chloride to the wash-water, as recommended above, dissolves out any fibrinoplastin that may adhere to the fibrin. In case of experimenting upon the blood of mammals, this addition of sodium chloride is of advantage only as it causes the fluid to filter more rapidly; for the amount of fibrinoplastin, which would remain undissolved with the fibrin in from 30 to 40 c. c. of blood, would not be sufficient to materially influence the weight. However, if the blood under examination be that of a bird or an amphibian, the fibrin should be well-washed with a solution containing from one to three per cent. of sodium chloride; then with water and alcohol.

In order for this estimation to be exact and easy of performance, it is necessary that the fibrin should be washed by decantation until the supernatant fluid is perfectly clear: for if the fibrin be brought upon the filter, before it has been well washed, the fluid will filter so slowly that the fibrin will partially decompose before it can be weighed.

#### SERUM. — *Plasma*

§ 67. It has already been mentioned that after plasma coagulates and as the clot contracts, a clear fluid separates and surrounds the coagulum. This is serum and is equivalent to the plasma minus the fibrin. It is colored partly by small quantities of dissolved hæmoglobin and partly by a coloring matter peculiar to itself. When examined with the spectroscope, the lines characteristic of hæmoglobin are observed. Serum has a specific gravity of from 1027 to 1030, has a more decidedly alkaline reaction than plasma and is coagulated on being boiled with mineral acids and many dilute metallic salts.

The various transudations, contents of cysts and synovial fluid resemble serum and the methods to be given for the examination of the latter will apply to all serous fluids. These, with an occasional exception, are more or less alkaline in reaction and vary in specific gravity from 1002 to 1030. Some are



viscid and can be drawn out into threads; while others are thin and contain much water. Some are perfectly clear, while others are colored with blood or bile, or are rendered turbid by the presence of epithelial scales, pus corpuscles, fat, threads of fibrin or crystals of cholesterin. Some of these morphological elements can be removed by decantation or filtration; while finely-divided fat can be removed only by repeated agitation with ether. These fluids contain albuminous substances, fats, extractives and organic salts. Upon microscopical examination, most serous fluids will be found to contain cytoïd corpuscles. The corpuscles of Gluge are not unfrequently found in the fluid contents of various tumors; these are larger than the white corpuscles of the blood, are granular in appearance and consist of coherent granules of fat, deprived of the cell wall. After removal from the body, some serous fluids coagulate spontaneously but slowly; while others do not coagulate at all. Of those of the latter class, coagulation may be caused in some by the addition of fibrin-ferment and in others by the addition of fibrinoplastin.

#### SEROUS FLUIDS.

§ 68. The *pericardial fluid* is of a yellowish color and, if it be removed immediately after the death of the animal, coagulates; but if it is not removed until some hours after death it does not coagulate spontaneously, but will do so after fibrinoplastin, prepared by the saturation method has been added. It is very rich in fibrinogen and contains about five per cent. of solids. Of the solid constituents, one-fourth is albuminous, and in structural diseases of the kidneys this amount is increased. In cases of excessive accumulation of this fluid, it has been found to contain crystals of cholesterin, uric acid and urea.

§ 69. *Hydrocele fluid* is an abnormal substance which sometimes accumulates in the serous sac of the testis. It is of a greenish-yellow tint, varies in specific gravity generally from 1010 to 1025 and contains from five to fifty per cent. of solids. Hydrocele fluid is rich in fibrinogen and seldom or never clots spontaneously, but does so after the addition of fibrinoplastin. In

some specimens, a peculiar kind of fibrinogen, which forms an easily soluble fibrin, is found. Sugar, urea and uric acid have been found in hydrocele fluid.

§ 70. *Peritoneal fluid* is found in that serous sac known as the peritoneum. Normally, the amount of this fluid is very small: but in ascites the accumulation is often considerable. The following remarks apply only to the pathological fluid: in appearance this fluid varies very much; sometimes it is clear and colorless, and at other times, it will be found milky and containing much finely-divided fat. It may contain urea, uric acid, xanthin, kreatin, cholesterin, lecithin, fat, and albuminous substances. In some cases, small moving parasites are observed. The specific gravity varies from 1005 to 1020.

§ 71. *Pleural fluid*, arising from certain pathological conditions, is either alkaline or acid in reaction; the acid fluid always contains pus and the acidity is probably due to the decomposition of the pus corpuscles. The specific gravity varies from 1005 to 1030; the specimens which contain pus are of greater specific gravity than those free from that morphological constituent. The gas, which collects in pneumothorax, is composed of  $\text{CO}_2$ , O, and N, with  $\text{H}_2\text{S}$  as an occasional constituent.

§ 72. *Cerebrospinal fluid* is clear, strongly alkaline and contains but a small amount of solids: consequently the specific gravity is low, from 1002 to 1007. It contains cholesterin, urea and mucin, also a substance which reduces copper, but which has not yet been isolated. Of the solids, only traces are organic; while the inorganic salts are represented by the chlorides, sulphates and phosphates of sodium and potassium.

§ 73. *Aqueous humor* is clear, of feebly alkaline reaction and does not coagulate either spontaneously or when heated; because it contains no fibrinogen and only traces of fibrinoplastin. Dr. Bence Jones found that after the administration of quinia and many other therapeutic agents, these could be detected in the humors of the eye. (See Lectures on Pathology and Therapeutics, p. 12 et seq.).

§ 74. *Synovial fluid* has a faintly-yellow tint, is alkaline

and contains mucin, albumen, extractives, fats and inorganic salts. The proportion of the constituents varies as the animal, from which the fluid is taken, has been quiet or moving. In the following table, taken from Hofmann's *Zoochemie*, the first column gives the composition of synovial fluid as taken from an ox which had been confined in a stall, and the second, of that from an ox which had been driven constantly :

	FIRST.	SECOND.
Water .....	969.9	948.5
Solids .....	30.1	51.5
Mucin .....	2.4	5.6
Albumen and Extractives.....	15.7	35.1
Fats .....	0.6	0.7
Salts.....	11.3	9.9

It will be seen from this table that the proportion of water is decreased by exercise. .

§ 75. *The Amniotic fluid* of the human subject is of a yellowish or brownish color, with a stale odor and a feebly alkaline reaction. This fluid is frequently turbid and on standing deposits a white flaky sediment, which on microscopical examination is seen to consist of epithelial scales. The specific gravity is variable, ranging from 1002 to 1030. It contains serum-albumen, fibrinoplastin, urea, kreatinin and occasionally sugar and ammonium carbonate, the latter probably arises from the decomposition of urea.

These various serous fluids have been mentioned in this place, because the methods to be given for the examination of serum will apply to the other serous fluids and in this way unnecessary repetition may be avoided.

#### EXAMINATION OF THE ALBUMINOUS SUBSTANCES IN SEROUS FLUIDS.

§ 76. The following methods are taken from Hoppe-Seyler's *Handbuch*. Besides serum-albumen, other albuminous substances, especially one or both fibrin-factors, are frequently found. In ovarian cysts, a caseous substance is present.

If both fibrinogen and fibrinoplastin be present and the temperature, reaction and contained salts be favorable, the

fluid will, sooner or later after its removal from the body, partially or completely coagulate: the fibrin thus formed will have the properties already described.

Whether coagulation takes place or not, it is necessary to test for the presence of globulins. Dilute a portion of the fluid with from ten to twenty times its volume of water and add, drop by drop, very dilute acetic acid as long as the precipitate increases; or it is better to treat the fluid after the addition of water and dilute acetic acid, with a stream of carbonic acid gas and allow to stand for some time. If the fluid becomes turbid on dilution and deposits a flaky precipitate on the addition of the acid, then it contains some substance which belongs to the albuminates or globulins.

Decant the supernatant fluid and heat a portion in a test tube; if it coagulates the fluid contains *serum-albumen*.

Suspend the precipitate, from which the greater part of the supernatant fluid has been decanted, in the remaining fluid and divide into two parts; to one of these add a few drops of a concentrated solution of sodium chloride; if the precipitate dissolves, the fluid under examination contains *fibrin-factors* or *myosin*. If the precipitate does not dissolve on the addition of the salt solution, it consists of *casein*.

To the second portion of the suspended precipitate add twice its volume of a one-tenth per cent. solution of HCl; if the precipitate dissolves it consists of *fibrin-factors*, *myosin* or *casein*.

To another portion of the fluid under examination, add a few drops of serum pressed from a recently-formed clot, shake and set aside in a warm place for a day, observing from time to time whether coagulation takes place or not. Should a coagulum form, it is evidence that the fluid contained *fibrinogen*.

To still another portion of the original fluid or to some of the precipitate (which has been thrown down on dilution and treatment with a current of carbonic acid gas) redissolved in dilute sodium hydrate, add some hydrocele fluid and allow to stand for one day. If a coagulum forms, *fibrinoplastin* is contained in the fluid.

Synovial, and some other fluids owe their viscosity to mucin or paralbumen. If mucin be present, the addition of acetic acid will throw down a precipitate which is not soluble in an excess of the acid, nor in a solution of sodium chloride. If paralbumen be present, the addition of acetic acid causes a turbidity which disappears on the further addition of the acid. As a confirmatory test for paralbumen, precipitate the fluid by the addition of three times its volume of alcohol, filter and dissolve the precipitate in water; if paralbumen were present, the aqueous solution would be viscid and would pass through a filter slowly.

§ 77. *Test for Sugar in Serous Fluids.*—Dilute the fluid with an equal volume of water, acidify with acetic acid, boil, remove the coagulum, which forms, by filtration and test the filtrate for sugar with Fehling's solution. If it be desired, the per cent. of sugar contained in the fluid may be ascertained as follows: Dilute a weighed portion of the fluid with water, acidify with acetic acid, boil, remove the coagulum by filtration, concentrate the filtrate on the water-bath to a syrup, extract this with boiling alcohol, filter, evaporate the alcoholic solution to dryness on the water-bath, dissolve the residue with water, and estimate the amount of sugar in the aqueous solution with Fehling's solution according to the method given for the estimation of sugar in the urine.

§ 78. *Test for Urea.*—Agitate a measured or weighed portion of the serum or other fluid with three times its volume of strong alcohol; collect the precipitate upon a filter; wash well with cold alcohol; evaporate the united wash-water and filtrate at a gentle heat on the water-bath; extract the residue with cold absolute alcohol and filter; evaporate this filtrate as before; dissolve this residue in a little water, and estimate the amount of urea in the aqueous extract with a standard solution of mercuric nitrate as directed for the quantitative estimation of urea in the urine. In order to confirm this estimation, collect the precipitate formed by the mercuric nitrate, on a small filter; wash with water; suspend the precipitate in water and treat with a current of  $H_2S$  gas; remove the precipitated mer-

cury sulphide by filtration; concentrate the filtrate on the water-bath to a small volume; place this concentrated solution in a strong tube, add a few crystals of pure barium chloride, and then a small quantity of a solution of barium chloride rendered alkaline with ammonia; the tube, which should not be more than half full, is now hermetically closed and heated on an oil-bath at  $200^{\circ}$  for four hours. The heat decomposes the urea, forming ammonium carbonate which precipitates the barium. The tube is now opened with a file, and the barium carbonate collected upon a filter, and well washed with water. The filtrate and wash-water are now discarded and the precipitate dissolved in dilute HCl. The barium is precipitated from this solution as a sulphate by the addition of dilute sulphuric acid. The mixture is heated and filtered while hot. The filter with its contents is dried, the barium sulphate and filter-ash placed in a weighed crucible, heated to redness, cooled over sulphuric acid and weighed; each part of barium sulphate represents .2574 parts of urea. (Hoppe-Seyler).

§ 79. *Test for Kreatin.*—Dilute some of the fluid, acidify with acetic acid, boil and filter. To the filtrate, add basic acetate of lead as long as the precipitate increases, but avoiding an excess of this reagent, and again filter. From the filtrate remove the lead by precipitation with  $H_2S$  gas and filtration. Concentrate the filtrate to a small volume and allow to stand for some days, when kreatin crystallizes. (For the appearance and further examination of the crystals, see under kreatin). This substance is abundant in the serum of typhus patients.

§ 80. *Test for Uric Acid.*—Remove the albumen by boiling and filtering through cloth; evaporate the filtrate to dryness; treat the residue with boiling water and filter while hot; concentrate this filtrate to a small volume; acidify strongly with acetic acid and allow to stand for a day or two when crystals of uric acid will be deposited and may be recognized by microscopic appearance and by the application of the murexid test. (See uric acid). Urates are in excess in the serum in instances of deficient oxidation, arthritis and valvular disease of the heart.

§ 81. *Test for Tyrosin and Leucin.*—To some of the fluid, add basic acetate of lead as long as a precipitate forms, but avoiding an excess, and filter; from the filtrate remove the lead by  $H_2S$  gas and filtration; concentrate this filtrate on the water-bath to a syrup and extract the residue with alcohol, and filter. The alcohol dissolves the greater part of the impure leucin and but traces of tyrosin. Evaporate the alcoholic solution and extract the residue with ammonium hydrate. To the ammoniacal solution add plumbic acetate as long as a precipitate forms. Suspend the precipitate, which consists of the oxide of lead and leucin in water; treat with  $H_2S$  gas and filter. Concentrate the filtrate when leucin crystallizes. In the residue, insoluble in alcohol, tyrosin forms in needle-shaped crystals. (See tyrosin and leucin). These substances are found in the serum, and transudates in cases of structural diseases of the liver.

§ 82. *Test for Bile-Acids.*—Remove the albumen with acetic acid and heat. Evaporate the filtrate to a syrup on the water-bath, extract with absolute alcohol and filter. Evaporate the filtrate; extract the residue with water to which a few drops of a dilute solution of sodium hydrate have been added; filter and to this filtrate apply Pettenkoffer's test. (See p. 41).

§ 83. *Estimation of the Amount of Albumen.*—To 100 c. c. of boiling water acidified with acetic acid, add gradually a weighed or measured portion of the fluid under examination. As the fluid is added the reaction of the boiling water should be tested from time to time, and if it becomes neutral or alkaline, a few drops of acetic acid should be added. Collect the albumen upon a weighed filter, wash with water, and then with boiling alcohol. Dry the filter with its contents at  $100^\circ$ , heat to  $120^\circ$ , cool over sulphuric acid and weigh. Repeat the heating, cooling and weighing until the weight remains constant.

§ 84. *Estimation of the Fat.*—Evaporate a measured or weighed portion of the serous fluid to dryness. Pulverize the residue and extract repeatedly with ether. Place the united ethereal solutions in a weighed dish, evaporate to dryness, cool and weigh.

§ 85. *Estimation of the Amount of Solids.*—A measured or weighed portion of the fluid is placed in a small weighed dish or crucible and evaporated to dryness at the temperature of the water-bath. The residue is kept for 12 hours at 100°, then heated to 110°, cooled over sulphuric acid and weighed. The heating, cooling and weighing are repeated until the weight remains constant. From this the per cent. of both the water and the solids may be calculated.

§ 86. *Estimation of the Inorganic Salts.*—If the residue, obtained as above and containing all the solids, be deprived of organic matter by burning and the ash be cooled and weighed, the per cent. of inorganic salts may be calculated. If it be desired to ascertain the amount of soluble and insoluble salts separately, the latter may be obtained by precipitating the albumen in a measured portion of the fluid, collecting the coagulum upon a Swedish filter paper (which has been deprived of inorganic matter by being first washed with dilute HCl and then with water until the wash-water has no longer an acid reaction), burning the filter with its contents and weighing the ash.

#### THE CORPUSCLES.

§ 87. Some reliable method of estimating the amount of corpuscles contained in specimens of blood has long been needed. Moreover, it is desirable to know the weight of the corpuscles in the moist, and not in the dry state. We wish to know the amount of the corpuscles as they exist in the blood and any method, by which the weight of dried corpuscles or of those changed either physically or chemically is obtained, is not the method desired. The efforts made in this direction by some of the most noted chemists have been more or less successful as will be seen from the following processes:

(1) To Hoppe-Seyler belongs the honor of introducing the first method here given for estimating the amount of the moist corpuscles. It is known that the corpuscles of the blood of mammals contain a trace of fibrin-forming substances, but the amount is so small that it may be overlooked and we may consider that the fibrin is furnished wholly by the plasma. Now, if the fibrin in a weighed portion of blood and that in a weighed



portion of plasma from the same blood be estimated, the weight of the plasma contained in a certain amount of blood may be calculated and this weight subtracted from the weight of the blood will give the weight of the corpuscles. Thus, suppose that  $b$  grams of blood yield  $c$  grams of fibrin and that  $b$  grams of plasma from the same blood yield  $d$  grams of fibrin; then  $b$  grams of blood contain  $\frac{c}{d}$  parts of  $b$  grams of plasma and if we represent the weight of the corpuscles contained in  $b$  grams of blood by  $x$ , its value is found from the following equation:

$$x = b - \frac{bc}{d}.$$

This method is applicable only to those specimens of blood which coagulate slowly and in which the corpuscles sink rapidly, as the normal blood of the horse, and that of men suffering with inflammatory disease.

The method is applied as follows: Draw a considerable portion of the blood from the vein into a cylinder surrounded by a mixture of ice and salt and another smaller portion, from 30 to 40 c. c., into the beaker for estimating fibrin (see p. 95), agitate, collect, wash, dry and weigh the fibrin as already recommended. After the corpuscles of the first portion have completely subsided, a small quantity of the plasma is transferred, by means of a cooled pipette, to the beaker, weighed and the amount of fibrin, which it contains, estimated.

It is necessary that the fibrin be estimated with the greatest care; for the amount of this substance obtained from blood is so small that a slight error in the estimation of it would be greatly magnified when the amount of plasma is calculated.

(2) This method consists in estimating the moist corpuscles from the amount of albumen and hæmoglobin which they contain. In this, four things are necessary; (*a*) the total amount of albumen and hæmoglobin in a certain portion of the blood must be ascertained; (*b*) the amount of albumen and hæmoglobin in the corpuscles is found; (*c*) the quantity of albumen contained in a given amount of serum must be known; (*d*) the

weight of the fibrin that can be obtained from a weighed portion of the blood is to be noted.

(a) Collect from 30 to 40 c. c. of the blood in a weighed dish or crucible, cover with a weighed watch-crystal, and weigh; then evaporate to dryness on the water-bath, transfer the residue to a mortar, washing the dish with alcohol and adding the washings to the contents of the mortar; rub up the mixture well and place in a beaker, washing all traces of the blood from the mortar into the beaker with alcohol; boil the mixture and collect the coagulum upon a small weighed filter, which has been freed from ash. Boil several successive portions of alcohol in the beaker and pour upon the same filter. The contents of the filter are washed with ether, then with distilled water and finally with boiling absolute alcohol. The filter with its contents is now dried at  $100^{\circ}$ , then heated to  $120^{\circ}$  for a short time, cooled over sulphuric acid and weighed. The heating, cooling and weighing are repeated until the weight remains constant. This (—the weight of the filter) gives the weight of the albumen + the hæmoglobin + the insoluble salts. The filter with its contents is now placed in a small, weighed, open dish and heated until all the organic matter is destroyed. The ash is cooled over sulphuric acid and weighed. By subtracting the weight of the ash from that of the albumen, hæmoglobin and ash, the weight of the first two in the blood is ascertained.

(b) A second portion of blood, from 30 to 40 c. c., is received in the fibrin apparatus, stirred, weighed, filtered through calico, diluted with 10 times its volume of a solution of sodium chloride, (made by mixing one volume of saturated sodium chloride solution with nine volumes of water). Allow to stand for 24 hours, or until the corpuscles have completely subsided; decant the supernatant fluid; wash the corpuscles once or twice by decantation with the salt solution; finally, transfer the corpuscles with a little water to a small dish; evaporate to dryness on the water-bath; rub the residue in a mortar with alcohol, and ascertain the amount of albumen and hæmoglobin as given under (a).

(c) A third portion of the blood, from 80 to 100 c. c., is

allowed to coagulate in a porcelain capsule; the separated serum is poured into a second dish, weighed, evaporated, dried, rubbed with alcohol and the amount of albumen estimated as in (a).

(d) The fourth portion, from 30 to 40 c. c., is collected in the fibrin apparatus, stirred, weighed, and the fibrin is estimated by the method already given.

In (a), we have found the weight of the albumen and hæmoglobin contained in the blood; in (b), the weight of the albumen and hæmoglobin in the corpuscles; in (c), the proportion of albumen in the serum; in (d), the amount of fibrin in the blood. It is now necessary to calculate each of these for the same weight of blood (100 grams). After having done this, it is easy to understand and apply the following principles: (1) the albumen + the hæmoglobin of the blood—the albumen + the hæmoglobin of the corpuscles = the albumen of the serum; (2) that after the proportion of albumen contained in a weighed portion of serum has been ascertained as in (c), the quantity of serum represented by the albumen calculated in (1) may be found; (3) that the fibrin + the serum = the plasma; (4) that the blood—the plasma = the moist corpuscles.

(3) There have been various methods proposed for numbering the corpuscles in a given volume of blood and thus ascertaining whether the proportion be normal or not. While this work deals with chemical and not with microscopical processes, it will not be amiss in this place to mention the most reliable method for the numeration of blood corpuscles. This consists in the use of Dr. Gower's modification of the *Hæmacytometer* of MM. Hayem and Nacet. The following is taken from Dr. Gower's description of the instrument, which has been furnished the author by the maker, Mr. Hawksley, of London:

"The *Hæmacytometer* consists of (1) a small pipette, which, when filled to the mark on its stem, holds exactly 995 cubic millimeters. It is furnished with an India rubber tube and mouthpiece to facilitate filling and emptying. (2) A capillary tube marked to contain exactly five cubic millimeters, with India rubber tube for filling, etc. (3) A small glass jar in which

the dilution is made. (4) A glass stirrer for mixing the blood and solution in the jar. (5) A brass stage plate, carrying a glass slip, on which is a cell, one-fifth of a millimeter deep. The bottom of this is divided into one-tenth millimeter squares. Upon the top of the cell rests the cover glass, which is kept in its place by the pressure of two springs proceeding from the ends of the stage plate.

"Various diluting fluids have been recommended in order to change as little as possible the aspect of the corpuscles. It is not well, however, to observe the characters of the corpuscles during the numeration. Whatever solution be employed, the corpuscles are more or less changed by it. One which answers very well is a solution of sodium sulphate in distilled water, of a specific gravity of 1025.

"The mode of proceeding is extremely simple. Nine hundred and ninety-five cubic millimeters of the solution are placed in the mixing jar; five cubic millimeters of blood are drawn into the capillary tube from a puncture in the finger, and then blown into the solution. The two fluids are well mixed by rotating the stirrer between the thumb and finger, and a small drop of this dilution is placed in the center of the cell, the covering glass gently put upon the cell, and secured by the two springs, and the plate placed upon the stage of the microscope. The lens is then focussed for the squares. In a few minutes the corpuscles have sunk to the bottom of the cell, and are seen at rest on the squares. The number in ten squares is then counted, and this multiplied by 10,000 gives the number in a cubic millimeter of blood.

"The average of healthy blood was decided by Vierordt and Welcker to be 5,000,000 per cubic millimeter, and later results agree with this sufficiently nearly to justify the adoption of this number as the standard, it being remembered that in a healthy adult man the number may be a little higher, in a woman a little lower. The number per cubic millimeter is the common mode of stating the corpuscular richness of the blood; but by employing this dilution, and squares of this size, a much more convenient mode of statement is obtained. Taking 5,000,000 as

the average per cubic millimeter for healthy blood, the average number in two squares of the cell is 100. These two squares contain .00002 cubic millimeter of blood, and it is proposed to take this quantity as the 'hæmic unit.' The number per hæmic unit, *i. e.*, in two squares (ascertained by counting a larger number, ten or twenty, and taking the mean) thus expresses the percentage proportion of the corpuscles to that of health, or made into a two-place decimal, the proportion which the corpuscular richness of the blood examined bears to healthy blood taken as unity. This is a much more simple method than any hitherto used. The proportion of white corpuscles to the red, or their number per hæmic unit, is best ascertained by observing the number of squares visible in the field of the microscope, and noting the number of white corpuscles in a series of ten or twenty fields. The number of red corpuscles corresponding to the ten or twenty fields is easily computed, and thus the proportion of white to red is ascertained. The normal *maximum* of white per two squares (hæmic unit) is .3."\*

#### WHITE CORPUSCLES.

§ 88. The proportion of white to red corpuscles varies as the animal fasts or eats. The author found that when his meals were taken at 8 A. M., 1 P. M. and 6 P. M., and no food was taken between meals, that the greatest scarcity of white corpuscles (ascertained from numeration of the corpuscles in a drop of blood from the finger) was apparent about 7 A. M., or just before breakfast, when the proportion of white to red was as 1 to 1800. The white were observed to be most abundant from 2 to 4 P. M., when the proportion was frequently 1 white to 200 red. In the blood of hibernating animals, examined about the close of the period of hibernation, the proportion has been observed to be one white, to many thousand red corpuscles.

The proportion between the white and red corpuscles varies in blood taken from different parts of the body. Hirt found in the arterial blood of the spleen 1 white to 2179 red corpuscles, and in the venous blood from the same organ 1 white to 70 red.

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\* For further details, see Practitioner, July, 1878.

In the hepatic veins the proportion is generally about 1 white to 180 red corpuscles. When the spleen is enlarged, the proportion of white corpuscles is generally greatly increased. I found in a case of this kind that the blood taken directly from the spleen, by means of a hypodermic syringe, two hours after a meal contained 1 white corpuscle to 15 red ones\*. The exact relation of the spleen to the white corpuscle is not understood; for it is well known that excision of that organ does not permanently influence either the absolute or relative number of either the white or red corpuscles.

In leucocythemia, besides the great abundance of white corpuscles, crystals consisting of double pyramids are not unfrequently observed. These may be mistaken for oxalate of lime, are insoluble in water, alcohol, ether and chloroform, soluble in acetic, tartaric and phosphoric acids and in alkalis. In dilute mineral acids, these crystals are very soluble, but in the concentrated acids, they do not dissolve but lose their firmness and can be flattened by pressure on the thin glass cover. In some instances, the points of the pyramids are drawn out into fine lines. These are known as the crystals of *Charcot-Neumann*, and their relation to the white corpuscles or their pathological significance is not known. In the case of enlarged spleen above referred to and in which I found the proportion of white to red corpuscles as 1 to 15 in the blood from the spleen, the same blood contained a great number of these crystals.

§ 89. *Chemistry of the White Corpuscles.*—The colorless corpuscles consist of a membrane enclosing a semi-fluid mass. On account of the facility with which many fluids pass through this membrane, the corpuscles swell, lose their granular appearance and often burst upon the addition of water or dilute acids. On the contrary, solutions of the caustic alkalis, alkaline salts, bile, and sodium taurocholate and glycocholate cause the corpuscles to contract and finally disappear. Fat is a constituent of the white corpuscle. Blood in leucocythemia is very rich in lecithin and the excess of this constituent

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\*See Michigan Medical News, March 25, 1878.

is contained in the white corpuscles; for the serum from such blood contains only traces of lecithin.

#### THE BLOOD IN DISEASE.

§ 90. It is necessary for the physician to know something of the condition of the blood in disease; for in this way, he acquires a more thorough knowledge of the nature of the disease and will be better able to base his treatment upon rational principles. It is true that this subject has not received the attention due it, but the intelligent physician will avail himself of what is already known, and endeavor to increase the number of facts by his original investigations. In cholera, the amount of the water of the blood is diminished by transudation from the capillaries of the intestines; moreover many inorganic salts pass out with the water; consequently we find the blood of the cholera patient poor in water and inorganic salts, and rich in corpuscles, albumen, fat and urea, and containing ammonium carbonate as an abnormal constituent arising from the decomposition of the retained urea. The quantity of fibrin is increased in inflammatory diseases, and in some cases as much as ten parts per thousand have been obtained. In some structural diseases of the liver, especially in the so-called yellow atrophy of that organ, tyrosin and leucin are contained in the blood, and may be obtained from the serum by the method already given. In puerperal fever, free lactic acid and bile-pigments are found in the blood; while of the normal constituents, the corpuscles, fibrin and albumen are increased. In diabetes, an excess of sugar is found in the blood; the sugar is not consumed, and when it accumulates in the blood, the excess escapes through the kidneys. In structural disease of the kidney, urea is found in the blood in excessive quantity.

The condition of the blood in various diseases is here represented in tabular form. The table slightly modified is taken from the *Lehrbuch* of Gorup-Besanez. The sign + represents an increase, and the sign — represents a decrease, while 0 is used when there is no characteristic variation in the constituent:

## CONSTITUENTS OF THE BLOOD.

DISEASES.	ABNORMAL CONSTITUENTS.	WATER.	FIBRIN.	CORPUSCLES.	ALBUMEN.	UREA.	SUGAR.	FAT.	SALTS.
Inflam. diseases	0	0	+	—	—	+	0	+	0
Acute Exant'm	0	0	0	—	0	0	0	0	+
Malaria.....	Bile-pigment.....	0	—	+	—	0	0	—	0
Morbus Brighti	0	0	+	—	—	+	0	+	+
Plethora.....	0	0	0	+	+	0	0	0	0
Chlorosis.....	0	+	+	—	0	0	0	0	0
Hydræmia.....	0	+	—	—	—	0	0	0	0
Puerperal fever	Bile-Pigment and free lactic acid.....	0	+	—	—	0	0	0	0
Pyæmia.....	0	0	—	white+	0	0	0	0	0
Cholera.....	Ammonia carbonate..	—	0	+	+	+	0	+	—
Dysentery.....	0	0	+	—	—	0	0	0	+
Atroph. of liver	Tyrosin and leucin....	0	0	0	0	0	0	+	0
Arthritis.....	Uric acid.....	0	0	0	0	+	0	0	0
Diabetes.....	0	+	0	0	0	0	+	0	0
TYPHUS:									
1. First stages...	0	—	+	+	+	0	0	0	0
2. Later stages..	0	+	0	—	—	0	0	0	+
Uremia.....	Ammonia carbonate..	0	0	0	0	+	0	0	0
Yellow fever...	0	0	0	0	0	+	0	0	0
Scurvy.....	0	+	+	—	0	0	0	0	+
Chyluria.....	0	0	0	0	0	0	0	+	0
Icterus.....	Bile-acids and pig'nts	0	0	0	0	0	0	+	0
Cancerous dys- thetica.....	0	0	+	0	0	0	0	0	0
Leucocythemia	Uric acid, hypoxan- thin, leucin, lactic and acetic acids and crystals of Charcot- Neumann.	0	0	white+	0	0	0	0	0

## EXAMINATION OF BLOOD STAINS.

§ 91. The reputation and in some instances the life of an individual depend upon the decision as to whether certain stains are produced by blood or by other coloring matters. Upon this subject several questions may arise and be of legal importance. These are (1) is the stain that of blood? (2) Is it the blood of man or of some of the lower animals? (3) Is it menstrual blood or not? These stains may be upon some article of clothing, upon wood, iron, dirt, grass, etc. In some instances the stains will afford abundance of material for examination; while in others only traces may be present, and these may be mixed with or covered by some other substances; thus



a stain which has been upon iron for a long time may be partially or wholly covered with rust.

*Hæmin Test.*—The most reliable test for blood in stains consists in an examination for the crystals of hæmin. This test is made as follows: The stains are first separated from the material on which they are deposited; if on iron or stone by scraping, if on wood by a sharp knife, if on cloth by rubbing with a little cold water. The dry stains or the residue obtained by evaporating the solution, (if cold water has been used to remove the stain) are covered with a small quantity of glacial acetic acid, boiled gently for a short time, then transferred to a watch-crystal and concentrated on the water-bath.\* If blood be present in quantities not too small, crystals of hæmin appear when the solution has been evaporated nearly to dryness. Under the microscope these appear as rhombic tablets of a reddish-yellow or brown color. These crystals are insoluble in water, alcohol, ether, chloroform, dilute hydrochloric, acetic, and phosphoric acids. In potassium hydrate they dissolve slowly, forming a brown solution which on standing becomes purple.

It is necessary to remember that in order to obtain the crystals of hæmin the blood must contain a small quantity of sodium chloride; now this salt is removed from dried blood with warm water; consequently if the stain has been washed with warm water before examination, it is necessary to add a small quantity of sodium chloride with the glacial acetic acid.

If the stain be upon cloth which has been washed with warm water, it is better to cut out a small piece covered by the stain, place it in a test tube, add glacial acetic acid and sodium chloride, boil, decant, or filter, and evaporate the solution on the water-bath, when, if blood were present, crystals of hæmin will be obtained. If the piece of cloth be thin and transparent, it may be placed on a glass-slide, a few grains of salt and a few drops of glacial acetic acid added, the whole covered with a thin glass, heated on the water-bath, and examined from time to time under the microscope. Should this test fail at first, more acid is added and the process repeated several times.

Crystals of hæmin after once being seen can hardly be mis-

taken for anything else. Cloth colored with indigo, when treated with glacial acetic acid as recommended above, yields crystals; but these are colored blue always, and are not at all similar in appearance to the crystals of hæmin. The greatest objections to the hæmin test for blood in stains are: (1) the crystals cannot be obtained when any substance is present which forms an insoluble compound with hæmatin; thus, they cannot be obtained when the stain has long been covered with iron rust; (2) they cannot be obtained when the stain is mixed with any decomposing matter, as with excrement. If a drop of blood be deposited upon a clean surface, hæmin crystals may be obtained from it many years afterwards; thus Scriba prepared crystals of hæmin in 1860 from blood-stains deposited on paper in 1820.

*Spectroscopic Test.*—Digest the stain on the cloth, wood, iron or stone with water to which a few drops of ammonium hydrate have been added. Bring the solution, filtered if necessary, before the spectroscope. If the characteristic lines of hæmoglobin appear, the presence of blood is certain. However, the non-appearance of these lines is not evidence sufficient of the absence of blood. It then remains to examine for hæmatin, and this examination is made as follows: To the ammoniacal solution, add glacial acetic acid sufficient to produce an acid reaction. Pour the solution into a graduated jar, add an equal volume of ether, shake well, and then allow the ether to separate. Should the ether not separate readily, add a little more of the glacial acetic acid. Remove the ethereal solution, which is of a brown color, and examine with the spectroscope; when if the stains were those of blood, the lines of hæmatin in an acid solution will be observed. These lines consist of a dark, sharply defined one in the red, and a less distinct one in the green.

*Test for Soluble Albuminates.*—Treat the stains with cold distilled water, when the coloring matter of the blood will be imparted to the water. Not unfrequently microscopical examination will reveal in the water fibres of undissolved fibrin. Upon heating some of the filtered aqueous solution, a cloudiness from the coagulated albumen appears. Another portion of the filtrate is precipitated upon the addition of nitric acid. Chlorine water

at first colors the solution green; but the further addition of chlorine destroys all the color and deposits a white flaky precipitate. If this precipitate be removed by filtration, and the filtrate be concentrated to a small volume, the test for iron will be obtained on the addition of potassium sulphocyanate. If some of the albumen, which has been coagulated by heat in the solution from the stains, be boiled with Millon's reagent, a brick-red color is produced.

If the stain has been washed with hot water, the albumen is coagulated, rendered insoluble, and fails to respond to the above test. If such a stain be washed with dilute sodium hydrate, the albumen is dissolved, and may be precipitated from the solution by the addition of nitric acid. The coloring matter is not imparted to the alkaline solution, consequently, if a stain from which the coagulated albumen has been removed by sodium hydrate be washed with hydrochloric acid, the coloring matter is taken up, and the concentrated acid solution responds to the test for iron when treated with potassium sulphocyanate.

Blood stains dissolve in boiling alcohol, which has been acidified with sulphuric acid, forming a solution which appears green by transmitted, and red by reflected light. The residue obtained by the evaporation of this solution also gives the test for iron, after the removal of organic matter by burning.

If to a mixture of ozonized turpentine (turpentine which has been exposed to the air for some time and which decolorizes water containing traces of indigo) and tincture of guaiacum of equal volumes, some particles from blood stains be added, the mixture is colored blue and a precipitate of the same color is deposited.

*Formation of a Ferro-cyanide.*—If some blood stains or an aqueous or alkaline solution of the same be evaporated to dryness with pure potassium carbonate and the dry residue be placed in a glass tube, more potassium carbonate added in the solid state, the tube be hermetically sealed and the contents heated to redness, potassium cyanide is formed. If now the tube be opened with a file and the fused mass be boiled with water and iron filings, the solution filtered and treated with a few drops of a solution of ferric chloride, Prussian blue will be produced. The

cyanide of potassium has been converted into the ferro-cyanide by being boiled with water and iron filings. This test is of value only as a confirmatory one and cannot be used when the stain is deposited upon leather or cloth. It is of most value when the stain is upon iron and mixed with rust; in this case, the addition of the iron filings is unnecessary.

It is impossible to distinguish between stains produced by blood from man and those produced by the blood of some of the domestic animals. It is true that the corpuscles of the blood of birds may be distinguished from those of the blood of man by microscopical examination; but the blood of the ox or dog cannot be distinguished with that certainty necessary in criminal prosecution from that of man. Moreover, in stains the corpuscles are frequently so altered that they can no longer be recognized by the microscope. If recent stains be moistened with a one per cent. solution of sodium chloride and a drop of the solution examined under the microscope, the corpuscles will be observed. *M. Barruel* is able to distinguish the animal from which a specimen of blood was taken by the odor obtained by warming the specimen with sulphuric acid. This is a degree of proficiency in the development of the sense of smell which but few can hope to attain and upon which none should wish to decide as to the guilt or innocence of a fellow being.

Menstrual blood can sometimes be detected by the presence of vaginal epithelium.

*Pfaff* claims to have discovered a means of ascertaining the relative age of blood stains. He depends upon the readiness with which the stains dissolve in a solution of arsenious acid (6 centigrams of the acid in 8 grams of water). Fresh stains dissolve immediately when treated with this solution. Stains which have been made for from one to two days require fifteen minutes for solution; while those from four to six months old require from three to four hours; and those over one year old, dissolve in from four to eight hours.

## LYMPH AND CHYLE.

§ 92. *Methods of Obtaining.*—(1) Lay bare the point of union of the jugular and subclavian veins and introduce a canula into the

thoracic duct. If the animal fasts the fluid thus obtained will be lymph, if recently fed, lymph mixed with chyle. (2) Lymph may sometimes be obtained by simply removing the epidermis and opening varicose enlargements of subcutaneous lymph vessels, especially of the thigh. (3) Lymph has been obtained in quantity sufficient for analysis from wounds.

(a). LYMPH.

§ 93. Lymph forms the return current from the tissue to the blood. It consists of a fluid containing certain formed elements. The former first appears in the radicles of the lymphatic vessels, while the latter originate for the greater part in the lymphatic glands. The lymph corpuscles are, so far as can be ascertained, identical with the colorless corpuscles of the blood. There are generally sufficient red corpuscles present to give to coagulated lymph a pink color. The lymph of the spleen has been found to be especially abundant in red corpuscles. Lymph contains some fat in a very finely-divided or molecular condition. These dust-like particles manifest the Brownian movement.

The fluid portion resembles in chemical composition either blood plasma or serum, as the fibrin-factors are present or absent. Of two portions of lymph taken from different vessels of the same animal at the same time, one may coagulate on standing, while the other does not; or two portions taken from the same vessel at different times may present a like difference. Lymph always contains less albumen in proportion to the inorganic, and easily soluble organic salts than blood plasma.

The following table represents the results of some analyses of human lymph:

PER CENT. COMPOSITION.	GUBLER AND QUEVENE.		SCHERER.	ODENIT'S AND LANG.	DÄHNHARDT AND HENSEN.
	I.	II.	III.	IV.	V.
Water .....	93.99	93.48	95.76	94.36	98.52
Solids .....	6.01	6.52	4.24	5.64	1.48 -
Fibrin .....	0.05	0.06	0.04	0.16	} 0.69
Albumen .....	4.27	4.28	3.47	2.12	
Fat, Cholesterolin and Lecithin.....	0.38	0.92	.....	2.48	
Extractives.....	0.57	0.44	.....	0.16	
Salts.....	0.73	0.82	0.73	0.72	0.79

## (b). CHYLE.

§ 94. *Properties*.—Chyle from the thoracic duct is an opalescent, milky, yellowish-white or pinkish fluid of a faintly-saline taste and faint but characteristic odor. Its specific gravity varies from 1012 to 1022, and its reaction is feebly alkaline. The formed elements are the same as those of the lymph, with the addition of much more suspended fat. The fat globules are larger than the fatty granules of the lymph. They are surrounded by albuminous envelopes and do not coalesce until the envelopes are destroyed. A few minutes after its removal from the body, chyle coagulates forming a white or pink coagulum, which after a few hours contracts and separates from the serum. The serum is opalescent with suspended fat.

The following table represents some of the most reliable analyses of chyle:

PER CENT. COMPOSITION.	MAN, BY REES.	HORSE, BY SIMON.	CAT, BY NASSE.	DOG, BY SCHMIDT.	ASS, BY REES.	COW, BY LASSAIGNE.
	I.	II.	III.	IV.	V.	VI.
Water.....	90.50	92.82	90.57	91.66	90.24	96.44
Solids .....	9.50	7.18	9.43	8.34	9.76	3.56
Fibrin.....	Trace.	0.07	0.13	0.21	0.37	0.10
Albumen...	7.08	4.98	4.89	3.58	3.52	2.80
Fat.....	0.92	0.49	3.27	3.30	3.60	0.04
Ext'ctives ..	.....	.....	.....	0.40	1.57	0.06
Salts .....	.....	1.14	1.14	0.84	0.71	0.57

## SPERMATIC FLUID AND STAINS.

§ 95. Spermatic fluid is of a white, grayish-white or yellowish-white color, of characteristic odor and neutral or alkaline reaction. The formed elements are spermatozoa, seminal corpuscles and epithelial cells. The chemical constituents are water, a casein-like albumen, phosphorus—containing organic bodies, and the same inorganic salts found in the blood, especially the phosphates of the alkaline earths. The fluid rapidly decomposes sufficiently to cause an abundant formation of crystals of ammonio-magnesium phosphate.

From the fresh fluid, especially on evaporation, groups of stellate crystals separate. These appear to belong to the mon-

oclinometric system and are, at least in part, organic, probably albuminous. They are soluble in water, but not in either alcohol or ether (Gorup-Besanez).

*General Appearance of the Stains.*—Spermatic stains are thin, of a grayish or yellowish color, and have irregular borders. If the stain is upon thin cloth it is visible upon both sides, while if upon thick cloth, only on one side. Spermatic stains upon linen have a glossy appearance and are translucent by transmitted light. The characteristic odor of semen is developed by moistening the stain with water or with vapor from a test-tube of boiling water; but this odor may be hidden by the presence of foreign substances.

*Effects of Reagents.*—Stains of pure semen dissolve in water forming a mucilaginous fluid which is not coagulated by heat, but is precipitated by alcohol, chlorine, mercuric chloride and lead acetate.

*Microscopic Examination.*—The identification of semen stains depends wholly upon microscopical examination. The appearance and chemical reactions of the stains are frequently so modified by the presence of foreign substances as to be valueless. Again the detection of the fecundating filaments (spermatozoa) is necessary before a stain can be pronounced spermatic. The method best suited for the microscopical examination of the stain is that first recommended by M. Robin, and is as follows: cut from the cloth a strip about 1 centimeter wide, including the entire stain if small, and its central portion if large, and extending both ends of the strip beyond the stain. Suspend this strip vertically with the lower end dipping into some distilled water in a watch-glass. By capillary attraction the whole stain becomes moist within from twenty minutes to three or four hours. With a clean spatula transfer the semen from the cloth to a glass slide, cover with a thin glass and examine with a microscope which magnifies from 400 to 600 diameters. If there be much mucus present, the addition of a drop or two of acetic acid will dissolve the mucus and thus render the detection of the spermatozoa more easy.

(Sometimes the examination must be conducted secretly and the cloth cannot then be cut. In such a case form a cone so

that the stain will cover the outer side, and then dip the apex, which should be free from stain, in the water and proceed as above).

Spermatozoa may be found entire or broken. When entire they vary in length from 0.040 to 0.045 millimeter. They consist of slender filaments with one extremity, the "head," presenting an enlargement which is oval and exhibits a double outline when seen under the microscope. The remaining portion is called the "tail." Sometimes the spermatozoa will be found broken, the break having occurred at the union of the "head" and "tail" or about the middle of the tail.

Besides spermatozoa, any or all of the following list of objects may be seen on microscopical examination.

- (1) Globules of oil.
- (2) Leucocytes or granular mucous corpuscles.
- (3) Seminal corpuscles which are irregularly rounded.
- (4) Crystals of ammonio-magnesium phosphate which are oblique prisms varying in length from 0.001 to 0.002 millimeter.
- (5) Epithelial cells from the urethra.
- (6) Substances from the cloth as cotton or woolen fibres, starch grains, etc.

### MILK.

§ 96. Milk is a white, bluish-white or yellowish-white fluid, of a sweetish taste, characteristic but pleasant odor and specific gravity varying from 1018 to 1045. The reaction of normal human milk is always alkaline; while that of carnivorous animals is constantly acid, and that of herbivorous animals (cow and goat) may be alkaline, neutral, or slightly acid. Milk is rendered opaque by the presence of suspended globules or milk corpuscles, which, as the milk is allowed to stand undisturbed, rise to the surface, presenting a more or less yellow layer of cream; while the underlying fluid becomes more watery and of a more markedly bluish tint. The formed elements are:

- (1) Milk globules, which appear spherical, have an average diameter of 0.0028 to 0.009 millimeter, and consist of fat.
- (2) Colostrum corpuscles, which are minute aggregations of



fat globules held together by an enveloping membrane. Sometimes a nucleus may be discovered. These corpuscles vary in size from 0.015 to 0.056 millimeter in diameter. They are abundant in colostrum, the secretion of the mother's breast from the first to the third or fourth day after the birth of the young, and are sparingly present in the normal milk of woman, but are very rarely found in the milk of the cow.

(3) Epithelial cells, which are not constantly present.

#### MILK GLOBULES.

§ 97. That the formed elements of the milk contain all the fat is not doubted, but whether the globules consist solely of fat or of fat surrounded by an albuminous envelope, is a question yet undecided. The following facts seem to support the prevailing belief in the existence of an albuminous envelope: (1) If milk be shaken with ether, little or no fat is dissolved by this agent; but if a few drops of a solution of potassium hydrate be first added to the milk, the ether will then take up all the fat and a transparent watery fluid will remain. It is generally supposed that the albuminous envelope is dissolved by the alkali, thus allowing the fat to be acted upon by the ether. (2) If milk globules under the microscope be treated with a drop of acetic acid, minute oily drops may be seen to exude from the corpuscles and collect, forming large irregular oil globules. It is supposed that the acetic acid gradually dissolves the membrane and allows the escape of the contained oil. (3) In the formation of butter by churning it is held that the albuminous elements are broken up by mechanical means.

On the other hand, many experimenters disbelieve wholly in the presence of an albuminous envelope. Soxhlet and others hold that the casein of the milk does not exist in a state of true solution, but in a highly distended condition, and to this fact the emulsive character of milk is due. This belief is supported by the fact that if milk diluted with water be poured upon a porous tile, the water, milk sugar and soluble salts will pass through the tile, while the butter and casein will remain upon the surface, from which they may be detached, dried and

weighed. Ether and sodium hydrate together destroy the emulsive character of the milk and then the fat is taken up by the ether. If milk be shaken with a solution of sodium hydrate, and then with chloroform or benzole, the fat will not be dissolved, and the portion remaining after the removal of the chloroform or benzole is still opaque, and microscopical examination will show that it contains the unbroken corpuscles in normal proportion. Since benzole and chloroform dissolve free fat as readily as ether does, it seems evident that there is a special action of the alkaline hydrate and ether, by which the emulsive properties of the milk are destroyed. Again, if but little acetic acid (not enough to cause any coagulation) be added to milk, which is then coagulated with a current of carbonic acid gas and afterwards agitated with ether, the ether will dissolve all the fat. In this case the amount of acetic acid is insufficient to dissolve the albuminous envelopes (did any exist) and the carbonic acid gas has no solvent power upon such membranes. Soxhlet supposes the formation of butter to be due to the passage of the fat from the fluid to the solid condition, caused by agitation; thus the fat in the milk may remain fluid at a temperature at which by itself it would solidify (just as water cooled below  $0^{\circ}$  may remain liquid).

#### COAGULATION OF MILK.

§ 98. (1) *Spontaneous Coagulation*.—If milk be left undisturbed, it becomes acid; or if acid when first obtained from the glands, its acidity gradually increases. This change takes place independently of the access of air. Milk placed in hermetically closed tubes and heated to  $100^{\circ}$  will remain fluid and unchanged in reaction indefinitely; but if the heating be omitted the lactose will be converted into lactic acid, and alcohol and carbonic acid will be formed. When the increase in acidity has progressed to a certain extent, the casein of the milk is converted into jelly-like masses (clabber); while a watery, sour, opalescent portion (whey) is formed. The coagulation of the casein takes place as soon as sufficient acid has been formed to convert the neutral alkaline phosphates into acid salts, and to remove the calcium phosphate from the casein. The cause

of the breaking up of the lactose into lactic acid is a ferment, which is precipitated from solution by alcohol and is weakened in its action by being boiled. If lactic acid ferment be obtained by precipitation with alcohol, and then be added to a solution of lactose, lactic acid will be formed. The spontaneous coagulation of milk is retarded by the addition of salicylic acid (1-5000 part of salicylic acid being sufficient to keep milk sweet for eight days), volatile oil of mustard (one drop to 20 c. c. of milk) and sodium bicarbonate.

(2) *Coagulation of Milk by Rennet*.—This process is wholly independent of the formation of an acid, it may take place while the milk is alkaline, and is due to a ferment present in the mucous membrane of the stomach. This ferment splits up the casein into at least two substances, cheese and whey-albumen. Cheese differs from casein, which separates on the spontaneous coagulation of milk, in many respects. The former is more difficult to dissolve in sodium hydrate and acetic acid. The ash of cheese contains calcium phosphate but no alkaline salts, while casein contains the latter. A solution of casein in calcium hydrate becomes a milky fluid without any permanent precipitation upon the careful addition of phosphoric acid to the point of neutralization; while a similar solution of cheese treated in the same way gives a permanent precipitate. That rennet coagulation is independent of the presence of lactose and the formation of lactic acid is shown by the fact that it occurs in milk which has been freed from lactose by dialysis, or in a solution of pure sodium-casein (Gorup-Besanez).

(3) *Coagulation by Acids*.—All the acids coagulate milk, but acetic and tartaric acids, when added in excess, dissolve the coagulum. The coagulation by acids, as in spontaneous coagulation, takes place as soon as the neutral alkaline phosphates are converted into acid salts and the calcium phosphate is removed from the casein. The coagulum consists of casein.

#### SPECIFIC GRAVITY OF MILK.

§ 99. It is frequently supposed that the purity of milk can be judged by its specific gravity. This is true only to a

limited extent. Of course, if a specimen of milk appears bluish and semi-transparent and has a low specific gravity, it may at once be discarded as poor or adulterated; but another specimen, which has a high specific gravity, may be no better or indeed may be worse than the first. The specific gravity of normal, unskimmed milk of the cow is about 1030 (from 1028 to 1033); while that of skimmed milk is about 1033 (from 1032 to 1036). Every time that ten per cent. of water is added the density decreases about three degrees; thus, pure milk to which one-tenth its volume of water has been added will have a density from 1029 to 1026, and that to which two-tenths its volume of water has been added will register between 1026 and 1023. This is true when water only is added. Removing the cream increases the density, and then if water be carefully added, the specific gravity may be made to correspond exactly with that of pure unskimmed milk. Again certain substances may be dissolved in milk and thus increase its density. The specific gravity may be ascertained with a hydrometer, lactometer or pycnometer.

#### HOPPE-SEYLER'S METHOD OF ANALYSIS.

§ 100. The following method is probably the best one known for the analysis of the milk of the cow or goat; but is not applicable in the analysis of woman's milk.

(a) After the milk has been shaken to secure equal distribution of the constituents, draw 20 c. c. of it from a burette into a graduated cylinder, and add water until the mixture measures 400 c. c. Pour into a sufficiently large beaker, add with stirring very dilute acetic acid drop by drop until a flocculent precipitate begins to fall. Now, treat for one-half hour with a current of carbonic acid gas. Allow to stand for from 12 to 24 hours. During this time the casein with the butter falls to the bottom of the beaker, and the supernatant fluid becomes transparent. Collect the precipitate upon a weighed filter, wash with water (preserving the united filtrate and wash-water), dry at  $110^{\circ}$ , cool over sulphuric acid and weigh. This less the weight of the filter gives the amount of casein and

butter in 20 c. c. of milk, and multiplied by 5 gives the amount of casein and butter in 100 c. c. of milk.

(b) Boil the clear reserved filtrate and washings, collect the albumen which is coagulated upon a weighed filter, wash with a little water (again preserving the filtrate and wash-water), dry at  $110^{\circ}$ , cool and weigh. This less the weight of the filter gives the amount of albumen in 20 c. c. of milk and multiplied by 5, the amount in 100 c. c.

(c) Place in a sufficiently large evaporating dish, 20 c. c. of Fehling's copper solution, add 80 c. c. of distilled water and heat. Measure accurately the reserved milk filtrate and washings, from which the casein, butter and albumen have been removed, and from a burette allow this filtrate to fall slowly into the boiling, dilute Fehling's solution, until the blue color of the latter is wholly destroyed and all the copper has been precipitated as cuprous oxide. The amount of the milk filtrate required to thus decolorize 20 c. c. of Fehling's solution is read off and contains .134 gram of milk sugar. From this the amount of lactose in the whole milk filtrate or in 20 c. c. of milk is easily calculated; and the amount in 100 c. c. of milk may be known by multiplying by 5. Thus, suppose that the united filtrate and wash-water measures 600 c. c., and 80 c. c. of this has been sufficient to reduce the 20 c. c. of Fehling's solution, then the 80 c. c. contains .134 gram of lactose and the amount of lactose contained in the total 600 c. c. may be found as follows:

$$80 \text{ c. c.} : 600 \text{ c. c.} :: .134 \text{ gram} : x - 1.005 \text{ gram.}$$

Since 20 c. c. of milk contains 1.005 gram of lactose, 100 c. c. of the milk would contain  $1.005 \text{ gram} \times 5 = 5.025$ .

(d) After shaking draw another 20 c. c. of the milk from a burette into a flask having a ground-glass stopper. Add an equal volume of the ordinary solution of potassium or sodium hydrate, then from 50 c. c. to 100 c. c. of ether. Stop the flask, shake well and allow to stand for one-half hour. Remove carefully the ether solution to a dry, weighed beaker. Add more ether to the alkaline milk, shake and remove the ether solution, adding it to that first removed. Repeat the agitations with ether

until a drop of the ether solution when allowed to evaporate, leaves no residue of fat. Evaporate the united ether extracts on the water-bath, dry at  $110^{\circ}$  in the air-bath, cool and weigh. This less the weight of the beaker equals the weight of the butter in 20 c. c. of milk, and multiplied by 5 gives the amount of butter in 100 c. c. of milk. The weight of the butter subtracted from the weight of the united butter and casein as found in (a) gives the weight of the casein.

(e) Into a weighed platinum or porcelain (preferably the former) capsule, pour 10 c. c. of the milk and again weigh. Heat in an air-bath at  $110^{\circ}$  until the weight remains constant. The loss in weight will show the amount of water contained in the milk. The solid residue of milk is very hygroscopic and therefore it should be cooled over sulphuric acid and in a covered crucible. The evaporation of the milk may be hastened by occasionally breaking up the pellicle which forms upon the surface. This should be done with a small platinum or glass rod which should be weighed with the crucible. During the evaporation of the milk, the residue will generally become brownish from slight decomposition of the lactose. Practically, the error thus caused is so slight that it need not be regarded. However, the milk may be evaporated with an air-pump over sulphuric acid. In this case there would be no decomposition of the sugar; but the time required is so great that this method is generally inapplicable.

(f) Heat carefully the residue obtained in (e) until all the carbon is oxidized. Cool and weigh the residue which consist wholly of mineral salts.

#### HAIDLEN'S METHOD OF ANALYSIS.

§ 101. Wash pure burnt gypsum with water, collect it upon a filter, dry at from  $105^{\circ}$  to  $110^{\circ}$  (not higher), and rub to a fine powder. Pour 20 c. c. of the milk to be examined into a small, weighed porcelain capsule and again weigh. To the milk add a weighed portion (from 1 to 3 grams) of the powdered gypsum. Heat the mixture over a small flame until it begins to boil, then continue the evaporation in the air-bath at from  $105^{\circ}$  to  $110^{\circ}$ , or dry over sulphuric acid by means of an air-pump, and

weigh. The loss in weight is that of the water of the milk; while the weight of the residue minus the weight of the gypsum gives the amount of solid residue.

(b) Rub the dry residue obtained in (a) to a powder in a mortar, transfer to a dry, weighed flask with a close fitting stopper (washing any little particles from the mortar into the flask with ether) add ether, close the flask and shake thoroughly. Allow to stand for some time, then filter through a weighed filter (allowing as far as possible the solid particles to remain in the flask). Repeat the agitation with ether as long as a drop of the filtered ether, when evaporated, leaves any residue. Then wash the contents of the filter with more ether (preserving the united ether filtrates). Dry the flask and filter in the air-bath and weigh both. The sum of these weights less the weights of the flask and filter, subtracted from that of the solid residue found in (a) gives the weight of the portion removed by ether or of the fat.

(c) Cover the contents of the flask with alcohol, heat to slight ebullition and pour through the same filter used for the ether extract. Repeatedly extract the contents of the flask with alcohol, pouring upon the same filter. Wash the contents of the filter with alcohol (preserving the united alcoholic filtrate). Dry and weigh the flask and filter as before. The sum of these weights subtracted from the sum of the weights obtained after extraction with ether gives the weight of the substances soluble in alcohol or of lactose and certain salts. Since lactose is but sparingly soluble in alcohol, a considerable amount of alcohol must be used and the extractions repeated as long as the filtrate leaves any residue on evaporation.

(d) Evaporate the alcohol extract on the water-bath, dry in the air-bath at  $110^{\circ}$  and weigh. This gives the weight of the lactose and certain salts soluble in alcohol, and the result here obtained is used simply in confirming or correcting that obtained in (c).

(e) Transfer the residue obtained in (d) by means of a little water to a small weighed porcelain capsule, dry, carefully incinerate, cool and weigh. This gives the weight of the mineral

substances extracted by alcohol, and this subtracted from the result obtained in (d) gives the amount of milk sugar.

(f) Evaporate on the water-bath the ether solution obtained in (b), dry in the air-bath at  $110^{\circ}$  and weigh. This gives the weight of the fat and confirms or corrects the result obtained in (b).

(g) In a second portion of the milk estimate the total amount of mineral salts as directed in Hoppe-Seyler's method. From this, subtract the amount of the mineral salts dissolved in alcohol. This gives the amount of salts remaining together with the gypsum, casein and albumen after the extraction with both ether and alcohol; and the subtraction of the weight of the salt + that of the gypsum from the weight of the total residue gives the weight of the casein + the albumen.

The special value of this method is due to the fact that a small amount of milk suffices for the estimation of all the constituents. Consequently the method is especially applicable in cases in which but a small quantity of milk can be obtained as in the analysis of woman's milk.

#### VOGEL'S OPTICAL ESTIMATION OF THE FAT.

§ 102. The degree of opacity of a specimen of milk is dependent upon the number of fat globules in a given volume: the greater the proportion of fat, the less transparent is the milk and *vice versa*. Upon this fact, A. Vogel has devised a method of ascertaining approximately the richness of milk, and the amount of water if any, with which the milk has been diluted.

The apparatus necessary is (1) a glass jar, with the height to which it is filled by 100 c. c. of fluid marked, (2) a pipette holding 10 c. c. or more and graduated to 1.5 c. c.; (3) a test-glass made of two parallel plates of glass just one-half centimeter apart and held in position by a close metallic frame.

Fill the glass jar exactly to the 100 c. c. mark with clear well water. Agitate the milk to be examined so as to equally distribute its constituents. By means of the pipette add 3 c. c. of the milk to the water in the glass jar. (If cream is being examined only 1 c. c. should be added to the water). Shake the mixture carefully and pour some of it into the test-glass,



placed in a darkened room. Observe, through the test glass and contained milk, the flame of a tallow candle placed one meter from the observer. If the contour of the flame is distinctly visible, return the contents of the test glass to the jar, add one-half c. c. more of the milk and test with the flame as before. Repeat the careful addition of milk until the contour of the flame is no longer visible. The total amount of milk added to the 100 c. c., in order to obstruct the light of the flame, is now recorded and the per cent. of fat is indicated in the following table:

C. C. OF MILK USED.	PER CENT. OF FAT.	C. C. OF MILK USED.	PER CENT. OF FAT.	C. C. OF MILK USED.	PER CENT. OF FAT.	C. C. OF MILK USED.	PER CENT. OF FAT.
1	23.43	7	3.54	16	1.68	45	0.74
1.5	15.46	7.5	3.32	17	1.60	50	0.69
2	11.83	8	3.13	18	1.52	55	0.64
2.5	9.51	8.5	2.96	19	1.45	60	0.61
3	7.96	9	2.86	20	1.39	70	0.56
3.5	6.86	9.5	2.77	22	1.28	80	0.52
4	6.03	10	2.55	24	1.19	90	0.48
4.5	5.38	11	2.43	26	1.12	100	0.46
5	4.87	12	2.16	28	1.06		
5.5	4.45	13	2.01	30	1.00		
6	4.09	14	1.88	35	0.89		
6.5	3.80	15	1.78	40	0.81		

This method is easy of application and is quite valuable for determining approximately the per cent. of fat; but is not applicable when the milk has been adulterated with substances (as starch and meal) which increase its opacity.

#### AVERAGE COMPOSITION OF MILK.

§ 103. The first table here given shows the average per cent. composition of some of the different kinds of milk. When the amount of albumen is not given, that substance was estimated with the casein.

	WATER.	SOLIDS.	CASEIN.	ALBUMEN.	BUTTER	LACTOSE.	SALTS.
Woman's Milk.....	88.91	11.09	2.91	.....	2.67	4.36	0.14
Cow's Milk.....	84.28	15.72	3.57	0.78	6.47	4.34	0.63
Goat's Milk.....	86.85	13.15	2.53	1.26	4.34	3.78	0.65
Ewe's Milk.....	83.12	16.88	4.19	1.91	5.37	4.49	0.92
Ass' Milk.....	89.01	10.99	3.57	.....	1.85	4.40	0.65
Sow's Milk.....	81.80	18.20	5.30	.....	6.00	6.07	0.83
Mare's Milk.....	90.31	9.69	1.96	.....	1.06	6.28	0.38
Camel's Milk.....	86.34	13.66	3.67	.....	2.90	5.78	0.66

The next table is taken from the Lehrbuch of Gorup-Besanez and shows the average composition and some of the physiological variations in the composition of woman's milk:

CONSTITUENTS IN 100 PARTS.	WOMAN'S MILK.						
	CLEMM.			VERNOIS AND BEQUEREL. Average of 89 Analyses	T. DY. Average of 14 Analyses.	CHRISTENN. Average of 5 Analyses.	BREL. Average of 6 Analyses.
	I. Fourth day after Birth.	II. Ninth day after Birth.	III. Twelfth day after Birth.				
Water.....	87.985	88.582	90.581	88.908	87.806	87.240	87.610
Solids.....	12.015	11.418	9.419	11.092	12.193	12.750	12.390
Casein.....	3.533	3.691	2.911	3.924	3.523	} 1.900	2.210
Albumen.....							
Butter.....	4.297	3.532	3.345	2.666	4.021	4.230	3.810
Lactose.....	4.118	4.298	3.154	4.364	4.265	5.960	6.090
Inorganic Salts....	0.209	0.169	0.194	0.138	0.285	0.280	0.280

## ADULTERATED MILK.

§ 104. The most common frauds practiced in the sale of milk are as follows:

- (1) The sale of skimmed milk for pure milk.
- (2) The addition of water to either skimmed or pure milk.
- (3) The addition of sodium acid carbonate to prevent or hinder coagulation of the milk.
- (4) The addition of starch or flour, etc.

The application of Vogel's optical test as given in § 102 is sufficient to determine whether or not the milk contains the normal amount of fat, when the fraud consists solely in the removal of cream or the addition of water. According to the experiments of Vogel, if it requires more than 6 c. c. of milk to obscure the flame, in his test, the specimen has undoubtedly been diluted with water or the cream has been removed. If cream is being examined the limit is 3.7 c. c.

The lactodensimeter of Quévenne is a convenient instrument for determining the specific gravity of milk. It consists of a hydrometer graduated from 1014 to 1042, taking the specific gravity of water as 1000. Normal milk never has a density greater than 1042, and milk diluted with 50 per cent. of water does not register less than 1014. On one side of the stem of

the lactodensimeter are indicated by words and figures the points to which the instrument will sink in pure milk and in that diluted with various proportions (1-10, 2-10, 3-10, 4-10, 5-10) of water. On the other side of the stem are similar words and figures indicating the depths to which the instrument will sink in skimmed milk, undiluted and diluted with various proportions of water.

*Bicarbonate of Sodium.*—A small amount of this substance is normally present in milk; but when an<sup>e</sup> excess has been added, the milk has a markedly alkaline reaction, a bitter taste and on evaporation leaves considerable residue of the salt. The amount of carbonate added can be determined by evaporating a given portion (50 c. c.) of the milk, extracting the residue with water, again evaporating and comparing the amount of carbon dioxide liberated on the addition of an acid with that from the residue of the same volume of normal milk, or with that liberated from 33 milligrams of sodium carbonate, which according to Marchandt, is the amount normally present in 50 c. c. of milk. Of course, this only represents so much carbonate and does not show the kind or amount of the base.

Chalk added to milk forms a deposit on standing. This deposit treated with hydrochloric acid liberates carbon dioxide and the calcium may be detected in the hydrochloric acid solution.

*Starch, Flour, etc.*—Boil a specimen of the milk, allow to cool, then add a few drops of tincture of iodine, when if starch be present the milk will be colored more or less of a dark blue. The starch grains should be identified by microscopical examination.

*Nervous Tissue.*—Evaporate 50 c. c. of the milk to dryness on the water-bath, extract the residue with ether, place the ether solution in a platinum crucible, evaporate to dryness and fuse this residue with potassium nitrate. Dissolve the fused mass in water and add barium chloride, when if nervous tissue had been added to the milk, there will be a precipitate of barium phosphate. The milk should be examined microscopically for fragments of nervous tissue.

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*Gum Arabic*.—A heavy white precipitate is produced by the addition of alcohol. *Gum Tragacanth*.—Shake the milk in a glass vessel then allow to rest, when small transparent lumps will be deposited upon the sides of the vessel. *Emulsion of Almonds*.—The specific gravity of the milk is not less than 1033. Examine with a microscope when minute globules, 1-400 millimeter in diameter may be observed. Add a few centigrams of amygdalin to two grams of the milk, when the odor of bitter almonds will be produced (Naquet).

#### DISEASED MILK.

§ 105. Milk may be abnormal from an excess or deficiency of normal constituents, or from the presence of pathological substances. Schlossberger observed an abnormally large amount of butter (28.54 per cent.) in the milk from an enlarged gland, which was afterwards amputated and found to weigh 14 pounds. The specific gravity of this milk was 0.98 to 0.99. Microscopical examination revealed no abnormal constituents.

Filhol and Joly examined a specimen of milk in which casein was wholly absent. It was not coagulable by either rennet or acetic acid, but did coagulate on being heated from 75° to 80°. The milk had an alkaline reaction, specific gravity of 1029 and presented on microscopical examination milk globules and colostrum corpuscles.

Gusserow found an excess of calcium in the milk of women with osteomalacia.

Many medicinal substances are found in milk. Iodine, iron and phosphoric acid especially have been found to appear in large quantities in the milk after their internal use.

The most important pathological constituents of milk are urea, blood, pus and animal and vegetable organisms.

Urea may be detected in milk by the method given for its detection in blood.

Blood may be recognized by change in color, or by microscopical or spectroscopical examination.

Pus in small quantity is likely to escape detection, but when present in large amount as in case of mammary abscess may be detected by microscopical examination.

Fuchs found in blue milk infusoria which he named *vibrio cyanogeneus*.

Gardner found in the blood and milk of a cow with the so-called milk-sickness (a disease prevalent in certain portions of Illinois, Indiana and Kentucky) bacteria resembling *bacilla subtilissima*. The same bacteria were found in the water of a spring in the neighborhood, also, in the blood of two persons affected with the disease.\*

#### OTHER SECRETIONS OF THE MAMMARY GLAND.

§ 106. *Colostrum*.—Colostrum, the secretion of the mother's breast from the first to the third or fourth day after birth, contains less lactose than milk, more albumen and has no casein. Its specific gravity generally varies from 1043 to 1060. The albumen gradually decreases, while casein appears and increases in amount until colostrum is replaced by milk. The colostrum corpuscles have already been described.

*Infant's Milk*.—The mammary glands of newly-born children secrete a fluid, which in chemical composition resembles milk diluted with water. It generally appears on the fourth day after birth, reaches its maximum quantity about the eighth day and disappears in the course of a month. Under the microscope are seen both milk and colostrum corpuscles. The Germans call this secretion witch-milk (Hexenmilch).

*Milk from the Glands of Men*.—In some rare instances, adult male mammals secrete a true milk.

#### MILK STAINS.

§ 107. It is sometimes desirable in cases of medico-legal inquiry to determine whether or not certain stains have been produced by milk.

Milk stains are yellowish when on white cloth. Wash the stain with a little cold water and examine with the microscope, when milk globules may be detected. The three following tests are considered sufficient to identify milk stains (Gorup-Besanez):

(1) To a part of the water extract, add a drop of acetic acid, if casein be present, a flocculent cloudiness, soluble in excess of

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\* Indiana Med. Reporter, volume I.

the acid, will appear. In the acid solution potassium ferrocyanide will produce a cloudiness.

(2) Extract a part of the stain with ether to which a little potassium hydrate has been added. Evaporate the ether on a watch-glass and examine with the microscope for fat globules.

(3) Extract another stain, if such be present, with fifty per cent. alcohol and test this extract, after evaporation of the alcohol and solution in water, for lactose with Fehling's solution.

## EPITHELIAL TISSUE.

### KERATIN.

§ 108. *Preparation.*—The epidermis, epithelium, horn, hair, nails and feathers constitute the class here known as epithelial tissue. All of these consist principally of a substance known as keratin. If some finely divided horn be washed first with boiling water, then with alcohol, ether, dilute hydrochloric acid and finally with water until the filtrate has no longer an acid reaction, keratin will remain. This is not a simple chemical compound, but probably contains several substances.

*Properties.*—Keratin is insoluble in alcohol and ether and is freed from fat by being washed with these reagents. In hot water, it swells but does not dissolve. When burnt, it gives off a characteristic odor, that of burning feathers. When heated with water or acetic acid in closed tubes to 200°, keratin dissolves and liberates  $H_2S$  gas; on cooling the solution forms a jelly-like mass. If hair, nails or horn-shavings be heated to 120° in closed tubes with glacial acetic acid, these substances are dissolved. This solution becomes turbid on the addition of water, is precipitated by neutralization with sodium hydrate and the precipitate is not soluble in an excess of the alkali.

Nitric acid colors most of epithelial tissues yellow and, on the application of heat, dissolves them forming yellow solutions and evolving nitrogen oxide. These solutions are changed to a brown color on neutralization with ammonia. By continued action of nitric acid upon epithelial tissue, oxalic acid is produced and may be obtained by neutralization and precipitation with some soluble salt of calcium, as the chloride.

Hydrochloric acid colors epithelial tissue violet, which after prolonged boiling is changed to brown, the tissue being dissolved. Hair, treated with concentrated hydrochloric acid, takes a purple color and dissolves after continued maceration in the cold.

By the action of warm concentrated sulphuric acid, most epithelial tissues are converted into a slimy mass, which on microscopical examination is seen to be composed of cells. On boiling this mixture, the tissue is dissolved. After prolonged boiling with dilute sulphuric acid, tyrosin and leucin are produced.

If horn-shavings be digested for a long time with concentrated potassium hydrate, a gelatinous mass is formed and is insoluble in the strong alkali in the cold. If the excess of alkali be removed by washing with water, the jelly dissolves in the dilute alkali. If acetic acid be added to this solution a white precipitate forms. By boiling epithelial tissue with alkalis, the sulphur of the tissue combines with the base forming an alkaline sulphide, and if to this, some hydrochloric acid be added hydric sulphide will be given off and may be recognized by its odor and by blackening silver.

In hair a granular pigment has been observed and these granules are wanting in white hair; this has led to the belief that the various colors of hair are due to physical and not to chemical properties. Be this as it may, pigments have been extracted from the colored feathers of some birds. Church has extracted the pigment from the feathers of the *Touracos*. The feathers are washed with ether and alcohol and then extracted with water containing one five-hundreth of an alkali. From this alkaline solution the coloring matter is precipitated on the addition of hydrochloric acid. The precipitate forms in layers of a deep-violet color; this coloring matter is not changed at a temperature of 100°, but above this point it melts, becomes dark-green and gives off violet fumes. It is known as *turacin* from the name of the bird from which it was obtained, and contains from 5 to 8 per cent. of copper.\*

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\* For Church's original paper on turacin, see Chem. News, 19, 265.

"The beautiful blue-violet wing-feathers of the Touracos lose their color when the bird gets wet and then give a red stain. On drying, they recover their original color or acquire a blue color if the bird has died in the interval. In the dead bird the coloring matter has become insoluble in water. When the feathers are soaked in ammonia-water and the filtrate is precipitated with acetic acid, the pigment is obtained as a red powder." (Gmelin's Handbook).

#### IDENTIFICATION OF HAIR.

§ 109. The medical expert is sometimes called upon to determine whether certain specimens of hair are from man or from some of the lower animals. Again criminals, in order to escape detection, may have their hair colored, and the expert is called upon to determine the original color.

*Nature of the Hair.*—Place the hair upon a glass slide, add a drop of glycerin, oil or some syrup, cover with a thin glass and examine with a microscope which magnifies at least 300 diameters. Human hair may be either cylindrical or flattened. It has a central canal or series of long cavities filled with coloring matter, and possesses the same diameter from end to end. The diameter of human hair varies from 0.015 to 0.09 millimeter. The hair is marked on its surface by slightly projecting scales having irregular borders and being separated from each other about 0.01 millimeter. These scales are transparent whatever the color of the hair.

The hair of the cow and horse never exceeds 12 millimeters in length and is largest at the root gradually tapering to the other extremity. It does not possess a central canal, is opaque and often presents enlargements from which project branches. The hair of some ruminants contains air cavities. Wool consists, however, of homogeneous hairs which are marked by numerous scales.

*Dyed Hair.*—The method of restoring the original color to hair which has been blackened will depend upon the nature of the dye that has been used. The most common methods of blackening the hair are the following:

- (1) The hair is first washed with water containing a little



ammonium hydrate, then with a neutral solution of some salt of lead or bismuth, and finally with a solution of hydrogen sulphide. After the application of the last solution the hair is allowed to dry. A black sulphide of lead or bismuth is formed. In other cases, the hair, after being cleansed with the yolk of an egg, is washed with a solution of calcium plumbate. Or a mixture of slacked lime, chalk and litharge is applied to the head, which is then left covered for some time with a warm cloth and finally washed first with dilute vinegar then with the yolk of an egg and water. The lead combines with the sulphur of the hair, forming a sulphide.

When the hair has been colored by this method, the original color may be restored by immersing it for three or four hours in a dilute solution of nitric or hydrochloric acid and the metal may be detected in the solution.

(2) The hair is first cleansed, then moistened with a solution of ammonio-silver nitrate (silver nitrate to which sufficient ammonia has been added to redissolve the precipitate which first forms), and allowed to dry in the sunlight. The silver is reduced and a portion of it is converted into a sulphide.

In order to restore the original color to hair thus dyed, immerse it for some hours in a dilute solution of potassium cyanide. If much silver sulphide has been formed the restoration will be only partial. Test the solution for silver.

(3) A method formerly much used but now almost wholly replaced by those already given is as follows: The hair is rubbed with a pomade containing charcoal dust. Hair thus dyed soils the hands and clothing for days after the application.

Wash the hair with ether until all the oil has been removed, then wash well with water, when the original color is restored.

*Bleached Hair.*—Dark hair may be bleached (any desirable shade of the blonde being obtained) by the action of chlorine gas. No method is yet known to restore the original color to bleached hair. If several days have elapsed since the bleaching, the original color may be detected by examining the hair near the scalp.

TYROSIN,  $\text{—C}_9\text{H}_{16}\text{NO}_3$ .

§ 110. Tyrosin is a product of the oxidation of the less complex animal tissues. It is prepared with facility from horn, nails, hair and the skin. Together with leucin, it is one of the products of normal pancreatic digestion. It is found preformed in the substance of the liver, spleen, kidneys, suprarenal capsules, thyroid and salivary glands in various degenerations of these organs. It is also found in diseased epidermis, thickened nails, and atheromatous cysts. It is a normal constituent of some insects. If cochineal be treated with boiling water, an amount of tyrosin equal to one-third of one per cent. of the cochineal is dissolved and crystallizes as the solution cools. Tyrosin represents a low state of organization and if the substance of any organ be unduly transformed into tyrosin, the function of such an organ cannot long be performed.

*Significance in the Urine.*—The tyrosin and leucin formed during pancreatic digestion are normally broken up in the liver into urea and uric acid. Any condition of the liver which prevents its normal action in this respect will allow the tyrosin and leucin to pass on unchanged into the general circulation and appear in the urine. Tyrosin and leucin have been found in the urine of yellow fever (Marvin), mitral and aortic incompetency, cirrhosis, acute rheumatism, nephritis, intestinal obstruction and cancer of the liver (Anderson).

*Preparation.*—All proteids can, by the action of oxidizing agents, yield tyrosin; but it is generally prepared from horn or hair. Boil two parts of horn-shavings with five parts of sulphuric acid and twelve parts of water for twenty-four hours. From time to time add water to replace that evaporated. While hot, dilute with water and saturate with marble dust or chalk. Filter, wash the precipitate with boiling water, in order to dissolve any tyrosin that it may contain, and unite the filtrate and wash-water. Concentrate on the water-bath, when tyrosin crystallizes in fine needles. In order to purify the tyrosin, redissolve in water, boil with hydrated oxide of lead and filter. (The compound of tyrosin and lead is soluble). Treat the filtrate with  $\text{H}_2\text{S}$  gas and remove the lead sulphide by filtration. Ren-

der the filtrate acid with acetic acid and concentrate, when tyrosin crystallizes.

*Properties.*—Tyrosin can be prepared from albumen, flesh, fibrin, and hair in the same manner as from horn. The crystals are fine, needle-shaped, often arranged in bundles. They are freely soluble in ammonium hydrate and in the dilute mineral acids, insoluble in acetic acid, alcohol and ether, very sparingly soluble in cold, more freely in hot water. These crystals are tasteless and odorless, but when burnt the odor of burning feathers is given off.

*Tests.*—(1) Heat tyrosin with an acid solution of mercuric nitrate, a rose color is produced, and a reddish precipitate is thrown down slowly. Boil finely-cut horn-shavings with the same reagent and observe that a similar color appears. (Hofmann's test).

(2) Dissolve tyrosin in concentrated sulphuric acid, warm on the water-bath; dilute with water, neutralize with calcium carbonate and filter. To the filtrate, concentrated if necessary, add neutral ferric chloride, when the solution is colored violet. (Piria's test).

(3) To some tyrosin on platinum foil, add two or three drops of nitric acid and gently heat to dryness. A bright yellow residue remains and dissolves in sodium hydrate, forming a reddish-yellow solution. (Scherer's test).

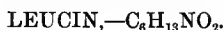
(4) Dissolve tyrosin in strong nitric acid and set aside until a yellow precipitate, nitrate of tyrosin, forms. This compound dissolves in sodium hydrate, forming a reddish solution.

(5) Dissolve tyrosin in strong hydrochloric acid and allow to stand until the chloride of tyrosin is deposited as an amorphous powder or in needle-shaped crystals. The chloride of tyrosin is soluble in alcohol.

(6) To a solution of tyrosin in ammonia add silver nitrate then neutralize with nitric acid, when argento tyrosin,  $C_9H_{10}AgNO_3$ , is deposited.

(7) To a saturated solution of barium or calcium hydrate add tyrosin, warm and set aside, when crystals, needles of the barium or calcium compound, are formed.

(8) Boil tyrosin with nitric acid for some time, neutralize and test the solution for oxalic acid. Tyrosin is easily converted by oxidizing agents into oxalic acid.



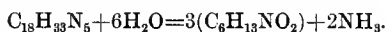
§ 111. Leucin is a constant product of decomposing albumen. It can be easily produced with tyrosin from the various substances mentioned in describing the latter. Leucin is formed with tyrosin in the method given for the preparation of the latter from horn.

*Preparation.*—(1) After the horn has been boiled the required time with sulphuric acid and water, the solution diluted, saturated with marble dust, filtered and concentrated, both tyrosin and leucin are deposited. For the separation of the leucin from the tyrosin and the subsequent purification of the former, Hlawitz and Habermann, as quoted by Hoppe-Seyler, give the following method: Boil the mixture with water to which a little ammonia has been added. To the hot solution add basic acetate of lead until a white precipitate forms and remains on being agitated. Filter, heat the filtrate to boiling, and saturate the ammonia with dilute sulphuric acid which also precipitates the excess of lead. Filter through a fast filter. As this solution cools, the tyrosin is deposited. Decant the supernatant fluid and treat it with  $\text{H}_2\text{S}$  gas to remove any traces of lead. Filter, to the boiling filtrate add an excess of recently precipitated hydrated oxide of copper and continue the boiling for a few minutes. A precipitate is formed and contains a part of the leucin. This precipitate is collected, suspended in hot water, treated with  $\text{H}_2\text{S}$  gas, acetic acid added and the copper sulphide removed by filtration. The filtrate is decolorized with animal charcoal and concentrated to a small volume. On standing, pure leucin is deposited. The other part of the leucin is in the blue solution which had been boiled with the oxide of copper. On evaporating this solution a blue colored compound of copper and leucin remains. If this compound be dissolved in water, the solution treated with  $\text{H}_2\text{S}$  gas and filtered, and the filtrate concentrated, leucin forms in needle-shaped crystals.

(2) Leucin exists preformed in the pancreas and is found in

the liver, kidneys and spleen in certain diseased states. In order to obtain the leucin, when preformed in any of these organs, the following process is used. Cut the organ into very fine pieces and rub the finely divided parts with ground glass in a mortar. Stir the pulp with much water, allow to stand for four hours, filter through a cloth and press the residue. Again wash with water and press. It is better if all the water can be removed by pressure in a screw-press. Unite the filtrate and wash-water, acidify with acetic acid, boil and filter in order to remove the albumen. Evaporate the filtrate to dryness, redissolve in a small volume of water. Separate from tyrosin and purify according to the method already given.

[3] When pure leucin is wanted, it is best prepared synthetically, as follows: Boil in a retort a mixture of two parts of valerian-ammonia, one part of hydrocyanic acid and an excess of dilute hydrochloric acid until the oily compound disappears. At first crystals having the formula,  $C_{18}H_{33}N_5$ , appear: then these take up water and are converted into leucin:



Evaporate to dryness on the water-bath. Treat the residue with a little water and neutralize with ammonia, when some leucin is precipitated; filter, collecting the precipitated leucin, evaporate the filtrate to dryness; treat the residue with dilute hydrochloric acid; concentrate on the water-bath and again neutralize with ammonia, when more leucin is deposited. Repeat the process as long as any leucin is deposited on neutralizing the solution with ammonia.

*Properties.*—When pure, leucin crystallizes ether in fine needle-shaped crystals arranged in bundles, or more commonly in thin, colorless rhombic plates. From solutions containing impurities, especially coloring matters, leucin is deposited, on concentration, in brownish balls or discs. In this form it is found in the urine, and the balls resemble urates, being distinguished from the latter by the weak refractive power of the leucin. The globules or balls resemble fat, from which they are distinguished by the insolubility of the leucin in ether. In some cases, the balls and discs will be seen to be composed of

radiating needles; in others of thin plates, and in others they will appear perfectly smooth.

Leucin is soluble in 27 parts of cold, more freely soluble in hot water. When the leucin is impure or when the water contains animal coloring matter, leucin is still more freely soluble. Thus, it is readily soluble in the urine. It is soluble in 1040 parts of cold, or in 800, of hot alcohol. When impure, it is freely soluble in spirits of wine. It is insoluble in chloroform and ether. Leucin dissolves freely in both alkalis and dilute acids: it dissolves in concentrated hydrochloric or sulphuric acid, without decomposition. From its solutions in acids, leucin is precipitated by neutralization.

*Tests.*—(1) Dissolve leucin to saturation in nitric acid and allow to stand. Fine needle-shaped crystals are deposited. The compound of leucin and hydrochloric acid is represented by the formula,  $C_6H_{14}ClNO_2$ , and forms in colorless plates. These compounds are freely soluble in water.

(2) Boil a solution of leucin with an excess of the hydrated oxide of copper; remove any undissolved copper, that may remain, by filtration; allow the filtrate to cool, when beautiful violet scales of  $3(C_6H_{13}NO_2) + 2CuO$  are deposited. The corresponding compound of leucin and mercury forms in white granules; while the lead compound appears in glistening white scales.

(3) Put some leucin into a perfectly dry test tube and gently heat. It is vaporized and deposited upon the upper and cool, part of the tube in thin plates often arranged in rosettes. If leucin be heated above  $170^\circ$  in a retort, a yellow, oily liquid is distilled over. On standing, this distillate is covered with crystals of ammonium carbonate. The leucin has been decomposed into amylamin and carbonic acid; later, the amylamin gives off ammonia, which combines with the carbonic acid.

(4) To some leucin, or the substance under examination and suspected to be leucin, on platinum foil, add a few drops of nitric acid and gently heat to dryness. If the substance be pure leucin an almost invisible residue remains. Warm this residue with a few drops of sodium hydrate, a more or less yel-

low color, according to the purity of the leucin, is produced. On farther concentration of the yellow sodium hydrate solution, an oily globule is produced and rolls about upon the foil without adhesion. (Scherer's test).

(5) Put some of the suspected substance into a dry test tube and gently heat, the peculiar odor of amylamin is given off, if leucin be present. On farther heating, the leucin is vaporized and deposited in crystalline plates upon the upper part of the tube.

(6) Treat a solution of leucin in hot water slightly acidified with nitric acid, with nitrous acid gas. A part of the leucin is converted into leucic acid. Evaporate to a syrup, extract the syrup with ether; evaporate the ethereal solution; dissolve the residue in water; filter; precipitate the filtrate with the acetate of zinc; collect the precipitate; wash with cold water; suspend in water and treat with  $H_2S$  gas. Remove the precipitated zinc by filtration, evaporate the filtrate to a syrup and allow to stand, when pure leucic acid is deposited in glistening, needle-shaped crystals.

*Leucic Acid.*—Leucic acid is freely soluble in ether, alcohol and water. Its solutions have a decidedly acid reaction. Dissolve some leucic acid in water and distribute the solution in six test tubes. To the first, add copper acetate; to the second, barium chloride; to the third, zinc acetate. These reagents throw down precipitates which, on standing, form in glistening crystalline scales. To the fourth, add calcium chloride; to the fifth, silver nitrate. The compounds produced with calcium and silver crystallize in needles. To the sixth test tube add lead acetate, when a white flocculent precipitate of lead leucate is formed.

Leucic acid melts at  $73^\circ$  and is sublimed unchanged under  $100^\circ$ . If some leucic acid be heated in a glass dish, on the water-bath, the sides of the dish, on cooling, will be covered with crystals. Leucic acid bears the same relation to leucin that glycollic acid bears to glycocoll and is represented by the formula,  $C_6H_{12}O_8$ .

## ELASTIC AND CONNECTIVE TISSUE.

## ELASTIN.

§ 112. The basis of elastic tissue is an albuminous substance known as elastin and is prepared as follows: Remove the cellular tissue from the *ligamentum nuchæ* of an ox or a horse. Boil the finely divided ligamentum for some time with a mixture of alcohol and ether in equal parts; decant the fluid and boil the pieces for 24 hours with water; then for another 24 hours with acetic acid and then with water until the rinsings are no longer acid. Now boil the substance with dilute potassium hydrate until the pieces begin to swell; decant the fluid; add to the residue water acidified with acetic acid and boil; again decant the fluid and wash well with water; add cold hydrochloric acid to the residue and allow to stand for 24 hours; pour off the acid and wash the residue with water as long as the wash-water leaves any residue on evaporation. The substance which remains insoluble, after being treated as above, is elastin. The fat has been removed by the alcohol and ether, and the collagen and inorganic substances by the water, alkali and acids.

Elastin prepared as above is of a yellowish-white color, elastic when moist, but brittle after drying. Examined under the microscope, the fibres are distinctly seen. It swells when boiled with water or acetic acid, but is insoluble in these reagents and in alcohol and ether. When heated with a concentrated solution of an alkali, elastin dissolves, forming a brownish solution, in which no precipitate is produced upon the addition of sulphuric acid. With pure concentrated nitric acid, elastin is colored yellow and converted into a jelly which upon the addition of ammonia becomes yellowish-red. When boiled for a long time with dilute sulphuric acid, elastin yields leucin and tyrosin, the former in larger quantity than the latter.

## COLLAGEN.

§ 113. The basis of ordinary connective tissue is collagen and is prepared as follows: Wash finely divided tendons with cold water; then cover with barium or calcium hydrate and



allow to stand for some days; then wash with water acidified with acetic acid and finally with water as long as the water dissolves anything.

Collagen is insoluble in cold water, but in boiling water it is converted into gelatin, and forms a jelly-like mass on cooling. Dilute acids and alkalis hasten the conversion of collagen into gelatin; thus, if collagen be placed in dilute acid or alkali until it begins to swell and then be placed in water at 40°, it will dissolve. In strong acetic acid, collagen swells and the fibres become indistinct, but reappear when the acid has been washed out with water or been neutralized with an alkali.

#### GELATIN.

§ 114. Boil collagen prepared as above, and allow the solution to cool, when gelatin will form; or, pure gelatin is best prepared by dissolving clean white pieces of isin-glass in dilute hydrochloric acid and removing the inorganic salts from this solution by dialysis, when pure gelatin remains.

Pure gelatin is an amorphous, transparent, yellowish-white, tasteless and odorless substance. In cold water it swells, but does not dissolve; in hot water it dissolves and is deposited in a jelly-like mass on cooling. It readily undergoes putrefaction and then gives off the odor of ammonia; putrefaction is prevented by carbolic acid. Gelatin heated in the flame swells, evolves the odor of burning feathers and burns with a pale flame.

From solutions in hot water, gelatin is not precipitated by nitric acid nor by acetic acid and potassium ferro-cyanide; but it is thrown down by chlorine gas, mercuric chloride and tannic acid. If an aqueous solution of gelatin be treated with a current of chlorine gas, it is precipitated in white, strong threads which contain chlorine and dissolve in the alkalis, forming chlorides. This precipitate evolves chlorine when treated with sulphuric acid. Alkaline solutions of gelatin give a violet color with Fehling's solution on boiling.

If gelatin be heated for a long time with water in sealed tubes at 140°, it is so modified as to be soluble in cold water. Neither the form soluble in cold water, nor that insoluble in the same menstruum is diffusible through animal membranes.

Fuming nitric acid dissolves gelatin, evolving nitrogen and forming oxalic and malic acids and fat. By prolonged boiling with dilute sulphuric acid or with alkalis, gelatin is decomposed and yields leucin and glycocoll.

### CARTILAGE.

§ 115. There are both histological and chemical differences between true or hyaline cartilage, and the fibrous variety or fibro-cartilage. The corpuscles of the former lie imbedded in a smooth, semi-transparent base; while the structure of the latter is distinctly fibrous: the basis of hyaline cartilage is *chondrogen*, while that of fibro-cartilage is *collagen*.

Chondrogen is changed by boiling water into a soluble substance which resembles gelatin in some respects and which is known as chondrin. It must be borne in mind that the organic basis of true cartilage is chondrogen, and that during the process of extraction this is changed into chondrin. Chondrin is prepared as follows: Boil costal cartilages from man or from calves for half an hour with water; remove with a knife the loosened perichondrium; macerate the cartilage in cold water for some time; then boil for four hours in Papin's digester at a temperature of 120°. or for 48 hours in an open vessel; filter the solution while boiling; to the filtrate add acetic acid, which throws down the chondrin; collect the precipitate and wash, first with ether and then with boiling alcohol, in order to remove the fat.

Dried chondrin is a glassy, transparent, yellowish substance, which is insoluble in alcohol and ether. In cold water it swells but does not dissolve, while in hot water it dissolves and separates as a jelly-like mass on cooling. It is also soluble in alkalis. Like gelatin, chondrin if heated in closed tubes for some time at 140° is so modified as to be soluble in cold water.

From its solutions, chondrin is precipitated (1) by dilute mineral acids, (2) by organic acids, (3) by many metallic salts. The precipitate produced by dilute mineral acids is soluble in an excess of the precipitant; the precipitates thrown down by strong sulphuric, arsenious and pyrophosphoric acids forming exceptions to this rule. Most of the organic acids precipitate

chondrin from its solutions; tannic acid causes only a faint opalescence. In the majority of cases the chondrin precipitated by organic acids is insoluble in an excess of the precipitant; that produced by acetic acid is sparingly soluble on being boiled with an excess of the acid. The fact that chondrin is precipitated by acetic acid affords an easy method of distinguishing between and separating chondrin from gelatin; for, as has been stated elsewhere, the latter is not precipitated by this acid. Moreover, the chondrin, precipitated by acetic acid, is soluble in either the ferro-cyanide or ferri-cyanide of potassium and in this way may be distinguished from albumen. Soluble salts of iron, copper, lead, silver and mercury precipitate chondrin from its solutions; the precipitate being soluble in an excess of either the precipitant or of the solution of chondrin. The deposit thrown down by the acetate of lead is insoluble; mercuric chloride only produces a faint cloudiness in solutions of chondrin.

§ 116. *Chondroglucose*.—It is a fact of no little interest that sugar can be obtained from cartilage; this sugar is lævorotatory, but differs both from dextroglucose and from lævoglucoſe (De Bary). It is prepared as follows: Cover finely divided pieces of rib-cartilage with cold dilute hydrochloric acid and allow to stand for some time, then pour off the acid; add more dilute acid and continue washing the cartilage with the acid until the inorganic matter is removed. Now boil the cartilage for some hours with concentrated hydrochloric acid; add to the mixture some recently precipitated lead oxide; boil again for a few minutes and remove the precipitated lead chloride by filtration. To the filtrate rendered alkaline by ammonia, add basic acetate of lead and collect the precipitate, which forms and contains the sugar, upon the filter; suspend the lead precipitate in water and treat with hydrosulphuric acid gas; remove the precipitated sulphide of lead by filtration and concentrate the filtrate, which contains the sugar, to a syrup.

Cartilage sugar readily reduces copper; but it is only partially fermentable. It seems very probable that two kinds of sugar are present; for before it is allowed to ferment at all, the solution turns the light—46.5°, and after fermentation is completed the

solution still reduces copper but turns the light only half as far to the left as it did previous to fermentation.

The resemblance between chondrin and gelatin is so close that the following table, taken from Hofmann's *Zoöchemie* and which points out the differences between these two substances, is inserted:

GELATIN.	CHONDRIN.
C=50.0    H= 6.7 N=18.1    O=24.6	C=50.0    H= 6.6 N=14.4    O=29.0
(1) Not precipitated by acetic acid.	(1) Precipitated by acetic acid.
(2) Soluble in mineral acids.	(2) Precipitated by mineral acids.
(3) Not precipitated by lead acetate.	(3) Precipitated by lead acetate and by most salts of the heavy metals.
(4) Precipitated by tannic acid and mercuric chloride.	(4) Only rendered turbid by tannic acid and mercuric chloride.
(5) Yields leucin and glycocoll by putrefaction.	(5) Yields leucin but no glycocoll by putrefaction.
(6) Yields no sugar on being boiled with hydrochloric acid.	(6) Yields chondroglucose on being boiled with hydrochloric acid.

Besides chondrin, cartilage contains water, fat and inorganic salts: the latter consisting of calcium phosphate and sulphate, magnesium phosphate and sodium chloride, carbonate, phosphate and sulphate. It is an interesting fact, first observed by von Bibra, that the salts of potassium are not found in cartilage. The per cent. of water contained in cartilage varies from 50 to 70. The per cent. of inorganic salts varies from 3 to 7 and seems to depend upon the age of the animal from which the cartilage is taken. The following table, taken from the *Lehrbuch* of Gorup-Besanez, shows the per cent. of ash found by von Bibra in the costal cartilages of persons of different ages:

A child of 6 months of age .....	2.24
A child of 3 years of age.....	3.00
A girl of 19 years of age .....	7.29
A woman of 25 years of age.....	3.92
A man of 20 years of age.....	3.40
A man of 40 years of age.....	6.10

Of the inorganic salts, calcium sulphate is the most abundant, constituting from 50 to 80 per cent. of the ash; the second salt in regard to quantity is calcium phosphate, which varies from 5 to 20 per cent. of the ash.

### OSSEOUS TISSUE.

§ 117. Bones consist of organic and inorganic matter and these can be separated by various means. Free bones as completely as possible from periosteum, blood-vessels and the contents of the medullary canal; crush into a coarse powder: extract with alcohol and ether in order to remove the fat; extract repeatedly with dilute hydrochloric acid (1 part of the acid to 9 of water) until the acid ceases to remove anything; wash the residue with water until the wash-water no longer has an acid reaction; boil the pieces thus freed from inorganic salts, with water for 24 hours; filter, while boiling, through a fast filter, wash any residue with boiling water; concentrate the united filtrate and wash-water to a small volume on the water-bath and allow to cool, when bone-gelatin is deposited. This substance will be found insoluble in cold, soluble in hot water and, in short, will manifest the properties already described as those of gelatin; while, should any chondrin be present it may be distinguished from the gelatin by precipitation of the former with acetic acid.

If bones be placed in dilute hydrochloric acid (1 part of the acid to 9 of water) and the acid be frequently changed, all the inorganic salts will be removed. The bone will still possess its original form, but becomes pliable and, if a long one, may be bent double or tied into a knot. On the other hand, if bones be kept at a red heat for some time, all the organic matter will be removed. The bone will maintain its original form, but will be brittle. In the bones of children the organic matter predominates and consequently their bones are not so easily broken.

The bones of the embryo even to the latest period of intra-uterine life contain no bone-gelatin or ossein but chondrogen; while after complete ossification, the bone contains no trace of

chondrogen. Freny found that the organic basis of some fish-bones and of the bones of certain water-fowls after being boiled with water, deposited no gelatin, and consequently differs from ossein.

Fossil bones contain that modification of collagen which is soluble in cold water and together with this, in some cases, the ordinary form, *i. e.*, that soluble in hot water and forming a jelly on cooling; the latter may be entirely replaced by the former. In very old fossil bones, the organic basis has entirely disappeared; also parts of the bone are replaced by silica and alumina, forming a petrification. Fresh bones when completely freed from blood and marrow contain no iron, but this element is often found in considerable quantity in buried bones. Haidinger found the medullary canal of the bones of a human skeleton containing crystals of vivianite.

The fat contained in bones has not been very thoroughly studied, but consists principally of triolein and tripalmitin. If it be desired, the amount of fat contained in bone may be estimated. For this purpose, extract a weighed portion of the dried bone-powder with ether; evaporate the ethereal solution at a low temperature or allow to evaporate spontaneously; again extract with ether, filter, evaporate the filtrate, dry the residue at 100° and weigh.

The marrow of the long bones consists of collagen containing fats. The cellular tissue of the spongy bones contains a soft, reddish substance, which consists of albumen, free acid and extractive matters. Whether the free acid be lactic, as claimed by Berzelius, is not yet positively known. Cholesterin is not unfrequently present in marrow, and hypoxanthin has been found in cases of leucocythæmia.

The inorganic constituents of bone are calcium chloride,  $\text{CaCl}_2$ , calcium fluoride,  $\text{CaFl}_2$ , calcium carbonate,  $\text{CaCO}_2$ , calcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ , and magnesium phosphate,  $\text{Mg}_3(\text{PO}_4)_2$ . From a great number of analyses made by Zalesky, it seems that there are certain variations in the proportion of organic and inorganic constituents, also of the various inorganic salts which are constant in different animals. The following table of

some of the analyses made by Zalesky is taken from the Lehrbuch of Gorup-Besanez :

IN 100 PARTS.	MAN.	OX.	TORTOISE, TESTUDO GRÆCA.	GUINEA- PIG.
Inorganic.....	65.44	67.98	63 05	65.30
Organic.....	34.56	32 02	36 95	34 70
Calcium phosphate.....	83.89	86.09	85.98	87.38
Magnesium phosphate.....	1.04	1.02	1.36	1.05
Calcium fluoride, chloride and carbonate.....	7.65	7.36	6.32	7.03
Carbonic acid.....	5.73	6.20	5.27	.....
Chlorine.....	0.18	0.20	.....	0.13
Fluorine.....	0.23	0 30	0.20	.....

In some diseased states, the proportion between the organic and inorganic constituents of bone may be very different from the normal and indeed not unfrequently the per cent. of the two is reversed; thus Marchandt found a femur in rachitis to contain 79.40 per cent. of organic and 20.60 per cent. of inorganic matter; Lehmann found a tibia, in the same disease, consisting of 66.36 per cent. of organic and 33.64 per cent. of inorganic matter; Ragsky obtained 81.12 per cent. of inorganic matter from a rachitic humerus; while Schloosberger ascertained that the amount of organic matter contained in the bones in three cases of craniotabes varied from 51.50 to 52.32 per cent., while the amount of inorganic salts in the same cases varied from 48.50 to 47.68 per cent.

In osteomalacia, not only is the proportion between the organic and inorganic salts abnormal, but the organic part is often radically changed so that after having been boiled with water it fails to deposit gelatin on cooling. Not unfrequently the bones in osteomalacia impart an acid reaction to water in which they are placed or with which they are washed. Schmidt and Weber claim to have detected free lactic acid in three cases of osteomalacia. Interesting in this connection is the assertion of Heitzmann, that by the continued incorporation of lactic acid in the food of dogs and cats, rhachitis and later, osteomalacia could be produced. However, these experiments have been repeated by Heiss and the above results are not confirmed. (Gorup-Besanez).

It is well known that the cavities of the bones of birds contain air and that the per cent. of inorganic salts, especially of calcium phosphate is greatly increased in these bones. On the other hand, the bones of fish are poor in inorganic salts and are rich in fat. Fish bones also contain salts of sodium and potassium, especially the sulphates and chlorides of these bases. The bones of amphibians contain less inorganic matter than those of mammals and more than those of fish.

The scales of fish have a composition similar to that of bones, the only difference consisting in a greater proportion of organic matter. The organic basis of fish scales is soluble in boiling water and forms a jelly on cooling. The so-called essence of pearl which is obtained from the scales of the white fish and which is used for the manufacture of artificial pearls consists, according to the analyses of Barreswil and Voit, of the carbonate of calcium and guanin. The scales of amphibians are essentially different from those of fish and belong both chemically and histologically to epithelial tissue.

How bones are formed and in what way they grow is a question of no little importance and one which is not yet fully understood. It seems that the chondrogen of the foetus is not transformed into ossein or collagen, but is replaced by it. We know but little more concerning the inorganic part of the bone. It has been proven that the chick as it escapes from the shell contains more lime than the interior of the egg and that the shell has, during the period of incubation, lost an equal amount of lime. How the lime is transferred from the shell to the embryo is not known. "The inner membrane of the shell, the interior parts of the embryo and in one case also the liquor amnii exhibited an acid reaction after fourteen days of incubation." (Lehmann). As the result of a number of experiments, it was found that the average amount of lime in one fully developed chick is five and a half times that found in the interior of one fresh egg (Bills and Vaughan).

Dr. Geo. G. Groff suggests that the solution of the carbonic acid, given off by the developing chick, in the fluids of the egg,



might possibly form a solvent for the shell; since calcium carbonate is soluble in water containing carbonic acid gas.

### TEETH.

§ 118. In the teeth three distinct structures exist; these are the dentin, cement and enamel. The first two of these contain the same inorganic constituents as bone and also yield an organic basis which dissolves in hot water and forms gelatin on cooling. The proportion between the organic and inorganic constituents of dentin is as 28 to 72.

The enamel is the poorest in water and richest in inorganic salts of any part of the body. The organic part of the enamel, when separated from the inorganic by solution of the latter in hydrochloric acid, appears as four- or six-sided prisms, which on being boiled with water do not form gelatin and which behave as epithelial tissue. The enamel of the growing teeth contains more organic matter than that of the fully developed tooth. The fluid which surrounds the tooth as it is enclosed in the dental sac is strongly alkaline in reaction and contains albumen. The watery extract of the enamel itself contains no trace of albumen: but if the inorganic salts be removed by nitric acid, the residue yields an albuminous substance which is precipitable by acids (Gorup-Besanez).

Saliva containing an excess of albumen or of other organic matter is very destructive to the teeth. The organic matter will collect to a greater or less extent between and around the teeth, where it undergoes an acid fermentation whereby the teeth are destroyed. In a case where the teeth were badly decayed, I found as much as 20 parts of albuminous matter per thousand in the saliva. Three or four hours after each meal, the contents of the mouth were slightly acid.

### FAT.

§ 119. Fat is an important constituent of many plants and animals. As an article of food its value can hardly be overestimated. The fat which is contained in animals is derived partially from the fat of the food and partially from the carbohydrates and the albumen of the food; thus the honey bee,

when fed entirely upon sugar, is able to produce wax, a substance closely related to fat in its chemical composition and physical properties; animals fed upon grain, potatoes, etc., substances rich in carbohydrates, soon gain more fat than is contained as such in the food; the carnivora often gain fat when food containing some starch is furnished them. This transformation of starchy substances into fat, or rather the derivation of the latter from the former, may take place outside of the animal body; thus Pasteur found that glycerin, a component of neutral fats, is produced together with alcohol and carbonic acid when cane sugar undergoes the alcoholic fermentation. Thus it is evident that carbohydrates supply fatty material as the result of chemical changes.

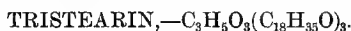
That fat results from certain changes in the albuminous molecule is evident; by the action of the pancreatic juice, leucin is produced from parapeptons; the unused muscle contains an excess of fat; in the aged and in certain diseased states various organs undergo fatty degeneration. Wetherill has shown that the dead body is converted into fats.

The small amount of oxygen contained in the fatty molecule and the fact that the hydrogen and carbon are converted into water and carbonic acid gas explain the value of fatty food in sustaining animal heat and show why it is so extensively used by the inhabitants of cold countries. Fat is a normal constituent of all the principal fluids of the body, with the exception of the urine, existing in a finely divided condition in the chyle, blood, milk, etc. In the solid tissue it is well distributed in the healthy state and in pathological conditions it may exist in excessive quantity in any or every organ. It represents a low state of organization and when the tissue of the liver, heart or other organ becomes unduly transformed into fat, that organ will soon cease to perform its function normally. The fat which accumulates pathologically is identical with that which, in smaller quantity, is a normal constituent of the tissues.

Fatty globules, even when present in small quantity, may be recognized by their microscopic appearance, They consist

of a thin membrane enclosing a fluid; in the dead body the contents of the membrane are sometimes found crystallized, in consequence of the removal of the heat of the body. These crystals generally appear in needles arranged in bundles or in rosettes. The perfect oil globule is spherical, floats upon water and is colorless or of a faintly yellow tint.

Some of the fats of the body are fluid and others solid at ordinary temperature. They give a neutral reaction, since they consist of fatty acids combined with glycerin forming neutral compounds. They are insoluble in water, sparingly soluble in cold, more freely in hot alcohol, and soluble in ether, chloroform and volatile oils; also soluble to some extent in each other, thus olive oil is a solution of tripalmitin and tristearin in triolein. Water containing albumen or bile-acid will hold fat in a finely divided state and will appear milky, while if fat be added to water alone the globules will float upon the surface. Upon being boiled with an alkali the fats are broken up into glycerin and fatty acids, the latter combining with the alkali to form a soap. If the fats, for instance butter, be allowed to stand exposed to the air, they sooner or later become rancid, volatile oils being formed. The most important of the fats of the animal body are *tristearin*, *triolein* and *tripalmitin*.



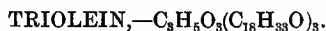
§ 120. It will be seen from the formula that tristearin is formed by the combination of three molecules of the mono-basic stearic acid with one of glycerin. Tristearin is prepared as follows: Extract mutton or beef tallow with cold ether, which dissolves only traces of tristearin; extract the residue insoluble in cold ether with hot ether and allow this extract to cool when tristearin is deposited in rectangular tablets or rarely in rhombic prisms. These crystals are very sparingly soluble in alcohol; they melt at  $63^\circ$

If tristearin, the melting point of which is  $63^\circ$ , be heated to  $64^\circ$  and then the heat be removed it solidifies at  $61^\circ$  and before it can again be melted, must be heated to  $66^\circ$ . Again if tristearin, the melting point of which is  $63^\circ$ , be heated to  $70^\circ$  and the heat be removed it solidifies at  $51^\circ$ , and, when again

heated, melts at  $52^{\circ}$ . There seem to be three modifications, the melting points of which are  $52^{\circ}$ ,  $63^{\circ}$  and  $66^{\circ}$ . (Hofmann).

*Stearic Acid*,  $\text{HO}(\text{C}_{18}\text{H}_{35}\text{O})$ .—If tristearin be boiled with sodium hydrate and the solution be diluted with 10 times its volume of water, or if ordinary soda soap be dissolved in hot water and then largely diluted with cold water, a precipitate will fall and will consist of the acid stearate of sodium, mixed with the acid palmitate of sodium, if soap has been used. This precipitate is treated with boiling alcohol and the solution decanted; when the solution cools, the acid stearate is again deposited and should be washed with cold alcohol and then treated with dilute hydrochloric acid. Sodium chloride is formed and the stearic acid set free; the former in solution is decanted and the latter is redissolved in boiling alcohol from which it crystallizes on cooling.

Stearic acid forms in thin plates, some of which are rectangular while others are oval. They are insoluble in water and cold alcohol; soluble in hot alcohol, ether, chloroform and benzole. They melt when pure at  $69.2^{\circ}$ , when mixed with palmitic acid, at a lower temperature.



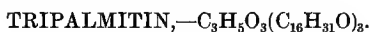
§ 121. Pure triolein is at ordinary temperature a colorless fluid, which on exposure to the air takes up oxygen and becomes more or less yellow. It is insoluble in water, slightly soluble in cold dilute alcohol, freely soluble in ether and absolute alcohol. It readily dissolves both tristearin and tripalmitin.

If olive oil be kept at or below  $0^{\circ}$  for 24 hours, a crystalline precipitate consisting of tripalmitin will form. The supernatant oily fluid may be decanted, dissolved in alcohol and again left for 24 hours at  $0^{\circ}$ , when the remainder of the tripalmitin is deposited. If now the alcoholic solution be poured off and diluted with water, triolein separates in globules, which crystallize in needles after being kept for some time at a temperature of  $-5^{\circ}$ .

*Oleic Acid*,  $\text{HO}(\text{C}_{18}\text{H}_{33}\text{O})$ .—Olive oil is freed from palmitin by being kept for 24 hours at  $0^{\circ}$  and the fluid oil is poured off, mixed with a small quantity of lead oxide and the mixture

heated for some hours at 100°. The oleate of lead, which is formed, is now dissolved in ether, while the other salts of lead remain insoluble in this menstruum. The ethereal solution is treated with a few drops of hydrochloric acid and shaken. Lead chloride is formed and upon standing sinks to the bottom. The supernatant ether containing the oleic acid is removed and evaporated at a gentle heat. The residue, which is impure oleic acid, is dissolved in ammonium hydrate and precipitated from the ammoniacal solution by barium chloride, as barium oleate. This precipitate is dissolved in warm absolute alcohol, from which barium oleate crystallizes on cooling. These crystals excluded from the air are treated with tartaric acid, which frees oleic acid. The fatty acid is washed quickly with water and kept in an atmosphere of carbonic acid until dry; this precaution is quite necessary, because oleic acid readily takes up oxygen from the atmosphere.

Oleic acid is a colorless, odorless and tasteless fluid, which when kept at a temperature of  $-4^{\circ}$ , crystallizes in thin plates. It is insoluble in water, freely soluble in ether, alcohol and chloroform. (Hofmann).



§ 122. It has already been stated that when olive oil is kept for some time at a temperature of  $0^{\circ}$ , tripalmitin is deposited in a crystalline form; these crystals, after the supernatant oil has been poured off, are dissolved in boiling alcohol from which they separate on cooling. They are slightly soluble in cold, freely soluble in hot alcohol and ether. From a saturated solution in hot alcohol, tripalmitin forms in needles as the solution cools. If stearin be also present the mixture not unfrequently forms in balls which consist of radiating needles or fine plates; this mixture has been mistaken for a fourth fat and designated by the name *margarin*. The crystals of tripalmitin melt at  $62^{\circ}$ .

#### DETECTION OF FATS.

§ 123. On account of their insolubility in water and solubility in ether, fats are easily separated from other substances when proper caution is used. Fats suspended in fluids may

be removed by agitating the fluid with ether, allowing to stand for a short time when the ethereal layer containing the fat will rise to the top and may be removed with a pipette. If it be desired to remove all the fat, the fluid may be repeatedly shaken with ether as long as the latter dissolves any fat; this is ascertained by allowing a few drops of the ethereal solution, placed on a glass slide, to evaporate, adding a drop of water to the residue and examining under the microscope for oil globules.

From emulsions, for example milk, fat is best removed by agitation with ether as above, after the addition of a few drops of sodium hydrate.

From fatty tissue or from solutions, fat is extracted as follows: Heat the tissue or solution at the temperature of the water-bath until all the water is driven off; rub up the residue with ether and remove the ethereal solution; boil the part insoluble in ether with alcohol; filter the alcoholic solution and evaporate it to dryness on the water-bath; extract this residue with ether; unite and concentrate the ethereal extracts which may contain besides neutral fats, fatty acids, cholesterin and coloring matters. In order to remove the fatty acids, evaporate the ethereal solution to dryness on the water-bath; add to the residue a small volume of a concentrated solution of sodium carbonate and again evaporate to dryness. The sodium carbonate does not saponify the neutral fats and these with cholesterin are removed by dissolving the residue in a little water, shaking this solution with ether and removing the ethereal layer. In order to separate the cholesterin from the fat, evaporate the ethereal solution at a gentle heat or allow it to evaporate spontaneously; heat the residue on the water-bath with an alcoholic solution of potassium hydrate and evaporate the alcohol; dissolve the residue in much water, shake with ether and remove the ethereal layer, which, if sufficient water had been added, contains only cholesterin. Heat the aqueous solution which contains the soap formed by the action of potassium hydrate on the fat, on the water-bath until all traces of any remaining ether are evaporated; slightly acidify the

solution of soap with dilute sulphuric acid, and allow to stand for a short time when the fatty acids are precipitated; filter, when the fatty acids remain upon the filter and the filtrate contains glycerin and traces of sulphates. Neutralize the filtrate with ammonium hydrate; concentrate to a small volume on the water-bath; extract with alcohol; filter and evaporate the alcoholic solution; rub up the residue with some lead oxide; suspend the mixture in water; treat with hydrosulphuric acid gas and filter. Evaporate the filtrate to a syrup when glycerin remains and may be recognized by its taste and by its dissolving copper oxide. (Hoppe-Seyler).

### MUSCULAR TISSUE.

§ 124. A chemical analysis of muscle is attended with many difficulties, some of which are anatomical, while others are physiological. In the first place the muscle must be freed as completely as possible from other tissues, as connective, elastic and nervous tissue, fat, blood- and lymph-vessels and the contents of these vessels. The blood is removed by injecting a one-half per cent. solution of sodium chloride until the returning current is colorless. When the inorganic constituents of the muscle are to be determined, a dilute solution of cane sugar is substituted for the one of sodium chloride. Other tissues are removed with the knife and scissors. The physiological difficulties are due to changes produced by various causes; thus, muscle at rest manifests a neutral or an alkaline reaction, while the tetanized muscle gives a distinctly acid reaction. Again as long as the muscle is contractile and living, it contains a fluid resembling the plasma of blood; while in the dead muscle, coagulation of this fluid has taken place. So long as the muscle is contractile, its plasma is transparent; while after the supply of blood has been cut off, the muscle becomes shorter, thicker, less elastic and less transparent.

### MUSCLE-PLASMA.

§ 125. *Preparation.*—Keep the contractile muscle of a frog, freed from blood by the injection of a one-half per cent. solution of sodium chloride, at from  $-7^{\circ}$  to  $-10^{\circ}$  until it

freezes; then cut it into fine pieces and rub these up in a mortar with snow containing one per cent. of sodium chloride. Soon the mass melts at a temperature of about  $-3^{\circ}$  into a cloudy, alkaline fluid, which filters slowly at a temperature below  $0^{\circ}$ . This opalescent fluid is muscle-plasma, and when exposed to an ordinary temperature is transformed into a jelly-like mass, which gradually contracts and presses out a fluid, muscle-serum.

#### MYOSIN.

§ 126. Myosin corresponds to the fibrin produced by the coagulation of the blood and, like fibrin, it is supposed to have its antecedents which exist in the plasma of the muscle. Myosin is not a constituent of living muscle, but is formed after death.

*Preparation.*—(1) Drop muscle-plasma, which has been kept in the cold, into water. As each drop falls, a fine white precipitate of myosin forms. This should be collected and washed with water. The myosin prepared in this way is quite pure.

(2) From dead muscle, the ready-formed myosin is separated as follows: The muscle, freed from blood, tendon, fat, fascia and connective tissue, is cut into fine pieces and washed with water until the wash-water no longer contains albuminous substances. The pieces are then rubbed up with a ten per cent. solution of sodium chloride and the viscid fluid, which forms, is filtered through linen. If now the filtrate be allowed to fall drop by drop into a large volume of distilled water, the myosin will be precipitated and may be collected and washed as above, or the mixture may be allowed to stand for several days when the myosin will have fallen to the bottom and may be freed from the supernatant fluid by decantation.

*Properties.*—Myosin forms in transparent flakes and is not at all fibrous. It forms very rapidly from muscle-plasma when the latter is subjected to a temperature of from  $35^{\circ}$  to  $40^{\circ}$ . Myosin is insoluble in water, soluble in sodium chloride solution of from five to ten per cent., and does not separate from these solutions on standing. From its solutions myosin is precipitated unchanged on the addition of much water. It is also



precipitated by boiling, and by alcohol; but by these it is changed into albumen and dissolves in alkalis forming albuminates. Myosin may be distinguished from fibrin by the insolubility of the former and the solubility of the latter in a solution of potassium carbonate.

#### MUSCLE-SERUM.

The fluid which separates after the coagulation of muscle-plasma, and which is known as muscle-serum, is of a faintly-yellow color, is neutral when kept at or below 0°; but at ordinary temperature it soon becomes acid owing to the development of paralactic acid. It contains an albuminate of potassium, an albumen which coagulates at 75° and another which coagulates at 45° and various extractives.

#### THE MUSCLE FIBRE.

The sarcous elements are supposed to be albuminous because they are affected like albuminous substances by most chemical reagents. They lose their transparency on being treated with acids or alkalis or by the action of heat. Muscle fibre, freed from myosin by being thoroughly washed with dilute solution of sodium chloride, is changed into syntonin by the action of dilute hydrochloric acid, into an alkaline albuminate by sodium carbonate. But the sarcous elements are not affected like other albuminous substances by alcohol. All other known albuminous substances, if insoluble in alcohol, are coagulated as by heat with this agent; while the sarcous elements of muscle are unchanged by alcohol. The same is true of the action of salicylic acid. It is possible that the sarcous elements consist of an albuminous substance united with another body, which is removed by alkalis and most acids, but not by alcohol and salicylic acid.

The entire albumen-content of muscle varies with the species of animal and the special muscle from 16 to 20 per cent. The proportions of the various albuminous constituents to one another have not been determined.

#### KREATIN, $\text{C}_4\text{H}_9\text{N}_3\text{O}_2$ .

§ 127. Kreatin is found in varying proportions in the mus-

cles of all vertebrates and of some invertebrates. According to Hofmann, the amount of kreatin in human muscle varies from 0.14 to 0.49 per cent. About the same amount is found in the muscles of the ox, dog and cat. A somewhat larger per cent. is present in the flesh of the domestic fowl and of the frog. Kreatin exists normally in small quantities in the brain, in blood, in the urine, and in various transudations.

Demant has shown\* that the per cent. of kreatin in the pectoral muscles of pigeons is trebled by depriving the animal of food for a period of eight days. This increase he supposes to be due to the following causes: (1) During starvation the flow of lymph is slow, and therefore the kreatin is not removed from the muscle as rapidly as in the normal condition. (2) More kreatin is formed on account of the consumption of the muscular tissue of the animal.

*Preparation.*—(1) Cut five pounds of muscle, freed from fat, into very fine pieces. Cover with water. Stir frequently for four hours and then filter through cloth. Wash and press the residue. Unite the filtrate and wash-water and boil quickly. Remove the coagulated albumen by filtration through cloth. To the filtrate add barium hydrate as long as a precipitate is produced. Remove the precipitated barium phosphate and sulphate by filtration. Treat the filtrate with a current of carbonic acid gas. Again filter, in order to remove the excess of barium which has been precipitated as a carbonate. Evaporate the filtrate to a syrup on the water-bath: if a pellicle forms on evaporation, it must be removed. Set the syrup aside in a cool place for a few days, when kreatin separates in rhombic prisms.

(2) Dilute Liebig's extract of meat. Remove the phosphates and sulphates by precipitation with barium hydrate and filtration and proceed as above.

*Properties.*—Kreatin crystallizes in beautiful prisms with many modifications. These contain one molecule of water of crystallization and are represented by the formula,  $C_4H_5N_3O_2 + H_2O$ . The crystals are sparingly soluble in cold, freely soluble in hot water. From a saturated solution in hot water, kreatin

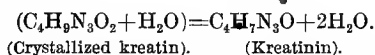
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\* Zeitschr. f. physiolog. Chemie, B. III, S. 380.

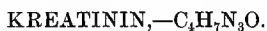
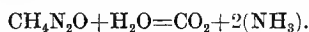
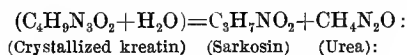
is deposited in fine needles on cooling. It is insoluble in cold alcohol and ether, soluble in hot dilute spirits of wine. Its solutions are neutral to litmus and have a bitter, irritating taste. If crystals of kreatin be heated to  $100^{\circ}$ , they lose their water of crystallization and become opaque.

Dissolve kreatin in dilute acid and allow the solution to evaporate spontaneously, when kreatin crystallizes unchanged.

Dissolve kreatin in strong hydrochloric, nitric or sulphuric acid and gently evaporate the solution. Crystals of a *kreatinin* salt are formed; the kreatin has given off water and been converted into kreatinin:



Boil kreatin with barium hydrate and observe that ammonia is given off. The ammonia may be recognized by the odor; and also by the production of a white cloud of vapor, if a rod moistened with hydrochloric acid be held over the boiling mixture. As soon as the ammonia is given off freely, cool the mixture, remove the barium with a stream of carbonic acid gas and subsequent filtration; evaporate the filtrate on the water-bath, when urea will remain and may be recognized by the formation of nitrate of urea on the addition of a drop of nitric acid. The kreatin has been converted into sarkosin and urea; while the latter has been decomposed into ammonia and carbonic acid:



§ 128. Kreatin is so easily converted into kreatinin, that it is not certain whether the latter exists preformed in muscle or not. The small amount of kreatinin which has been obtained by some chemists from flesh might have been produced from kreatin during the process of separation. Kreatinin is a constant constituent of normal urine.

*Preparation.*—It is best prepared from kreatin. Boil kreatin for an hour with dilute hydrochloric acid, evaporate to dryness on the water-bath, and redissolve the residue, which consists of

the chloride of kreatinin, in water. To this aqueous solution add some hydrated oxide of lead; boil, filter, and again evaporate to dryness on the water-bath. Extract the residue with alcohol and evaporate the alcoholic solution on the water-bath, when pure kreatinin remains.

Kreatinin may be obtained from kreatin by the action of other acids. Heat kreatin with dilute sulphuric acid on the water-bath for one hour. Neutralize the solution with barium carbonate, filter and evaporate the filtrate until kreatinin crystallizes.

*Properties.*—Kreatinin forms in prisms which belong to the monoclinometric system. It is more freely soluble in water than kreatin is; kreatinin requiring only 11.5 parts of cold water for solution. It is sparingly soluble in cold alcohol, freely soluble in hot alcohol. From its solution in hot alcohol, kreatinin crystallizes on cooling. Its solutions have a caustic taste resembling that of ammonia and give a decidedly alkaline reaction. Kreatinin is a true animal alkaloid, combines with acids forming salts and liberates ammonia from its combinations.

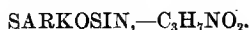
To a moderately concentrated solution of silver nitrate, add kreatinin; a dense precipitate of fine acicular crystals is formed. Boil the mixture, when the precipitate dissolves; but again separates on cooling. The precipitate consists of kreatinin-silver nitrate. A similar compound is formed by the addition of kreatinin to a solution of mercuric chloride.

To an alcoholic solution of kreatinin add a few drops of a neutral, concentrated solution of zinc chloride. A precipitate of the double chloride of kreatinin and zinc,  $(C_4H_7N_3O)_2 Zn Cl_2$ , is produced. This precipitate forms either in fine needle-shaped crystals, or in warty granules. Often, microscopic examination will show that the granules are composed of radiating needles. This compound is insoluble in cold water and alcohol, soluble in hot water and the mineral acids. If this salt be decomposed by ammonium sulphide, a part or all of the kreatinin is transformed into kreatin.

*Preparation from Urine.*—Kreatinin may be obtained from the urine and the amount daily excreted estimated by the fol-

lowing process which is known as Neubauer's method: To 300 c.c. of urine add milk of lime until an alkaline reaction is produced; then add calcium chloride as long as precipitation continues. Allow to stand for two hours; filter; wash the precipitate with water; unite the filtrate and wash-water, and evaporate to dryness on the water-bath. Mix the residue with strong alcohol (absolute or 95 per cent.). Pour the mixture into a clean beaker which has been rinsed with alcohol; allow to stand for six hours; at the expiration of this time a precipitate will have formed; filter the supernatant fluid; then collect the precipitate upon the same filter and wash with a small quantity of alcohol; unite the filtrate and washings. If these measure more than 50 c.c., concentrate to that amount with gentle heat on the water-bath. To the concentrated fluid add .5 c.c. of an alcoholic solution of perfectly neutral zinc chloride, of sp. g. 1.2. Stir the mixture vigorously until a cloudiness appears, then cover it with a glass plate and set aside in a cool place for four days; collect the crystals of kreatinin-zinc chloride on a weighed filter; wash with alcohol until a colorless filtrate appears and no longer gives the reaction for chlorine; dry the crystals on the filter at 100° and weigh. The normal amount of kreatinin excreted daily in the urine varies, according to Neubauer, from 0.6 to 1.3 grams. (Hoppe-Seyler).

It will be seen from a study of the sources of kreatin and kreatinin, that the amount of these substances present in the body and in the excretions will vary greatly with the kind of food. Liebig found that a dog, while being fed upon muscle, excreted large quantities of kreatin and kreatinin and but little kynurenic acid; while when the animal subsisted upon fatty food, the proportion of these substances was reversed. Since muscle contains kreatin, it is evident that an increased consumption of this article of food will augment the amount of kreatinin excreted in the urine; but as has been stated the per cent. of kreatin contained in muscle is increased during starvation.



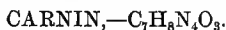
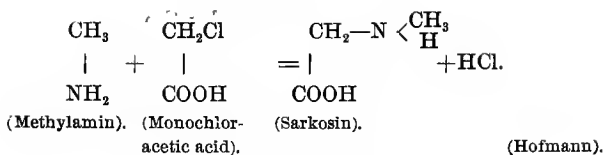
§ 129. The formation of this substance from kreatin has

already been referred to, and the reaction by means of which sarkosin and urea are produced from kreatin has been written. It is not itself a constituent of muscle and is of interest in this connection on account of its derivation.

*Preparation.*—Boil a saturated solution of kreatin with 10 times its volume of barium hydrate as long as ammonia is given off and barium carbonate is formed. (If it is necessary more barium hydrate may be added from time to time). As soon as ammonia is no longer given off, filter, treat the filtrate with a current of carbonic acid gas and remove the precipitated carbonate by filtering again; concentrate this filtrate on the water-bath to a syrup and allow to stand for some days, when sarkosin forms in crystals. In order to purify the crystals, dissolve them in dilute sulphuric acid, filter and concentrate this filtrate to a syrup on the water-bath. Wash this syrup with alcohol, then dissolve it in water, add barium carbonate and heat as long as carbonic acid is given off; remove the barium sulphate by filtration; concentrate the filtrate to a syrup on the water-bath and allow to stand for 24 hours when pure sarkosin crystallizes. (Hofmann).

*Properties.*—Sarkosin forms in large, colorless, rhombic prisms, which are soluble in alcohol and in water, not soluble in ether. It acts as a base uniting with acids forming salts. With gold chloride it forms a double salt which is freely soluble in alcohol and hot water, but very sparingly soluble in cold water. From its solution in hot water, this salt forms in rhombic tablets on cooling. With platinum chloride, sarkosin forms a double salt which crystallizes in large, yellow octohedrons.

Sarkosin is methyl glycocoll and can be formed synthetically by adding an excess of monochloracetic acid to an aqueous solution of methylamin and keeping the mixture at about 130° for some time; then removing the chlorine by silver oxide, decolorizing the solution with animal charcoal, concentrating to a syrup and allowing to stand for a few days, when sarkosin crystallizes. The formation of sarkosin synthetically is represented by the following equation:



§ 130. This substance has been found, as yet, only in the prepared meat extracts, in which it exists in as great a proportion as one per cent.

*Preparation.*—To Liebig's extract, add six times its weight of warm water; to this solution add a saturated solution of barium hydrate as long as the precipitate increases and then filter through linen; to the filtrate add basic acetate of lead and collect the precipitate which forms and consists of inorganic salts of lead, especially the chloride, and a double salt of lead and carnin; wash this precipitate with hot water, which dissolves all the carnin compound and only traces of the inorganic salts; treat the filtrate, while yet hot, with hydrosulphuric acid gas and remove the precipitated lead sulphide by filtration; concentrate the filtrate and add to it a concentrated solution of silver nitrate. This forms a precipitate which consists of silver chloride and a double nitrate of silver and carnin. Collect this precipitate and wash it, first with water and then with a small quantity of ammonium hydrate. The ammonia dissolves the silver chloride, while the nitrate of silver and carnin remains. Suspend this remaining precipitate in water and treat with a current of hydrosulphuric acid gas; heat the mixture and filter while hot; concentrate the filtrate and allow it to cool when carnin, more or less colored with impurities, crystallizes. It may be purified by solution in hot water and filtration of the hot solution through animal charcoal; but a part of the carnin will remain in the charcoal.

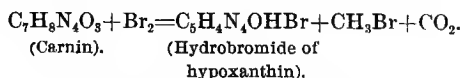
*Properties.*—Carnin forms in fine, irregular crystals, which are very sparingly soluble in cold, more freely in hot water, insoluble in ether and alcohol. Its hot aqueous solution is neutral in reaction and is not precipitated by the neutral acetate of lead; indeed the presence of the neutral acetate will

prevent the precipitation of carnin by the basic acetate of that metal. When heated to 100°, carnin gives up its water of crystallization and is transformed into an amorphous mass.

If carnin be dissolved in warm hydrochloric acid, crystals of carnin chloride form in needles as the solution cools. This compound is formed by the simple combination of the acid with the base, and has the formula,  $C_7H_8N_4O_3.HCl$ . If platinum chloride be added to a solution of carnin chloride, a double salt is formed and deposited in a yellow crystalline powder. Silver nitrate precipitates carnin, forming a white flocculent mass which has the formula,  $(C_7H_8N_4O_3)_2AgNO_3$ , and is insoluble in both ammonia and nitric acid.

If a small quantity of carnin be treated with fresh chlorine water and a trace of nitric acid and the mixture, after gas has ceased to be given off, be heated to dryness on the water-bath, a white residue remains. If now the dish containing this residue be placed under a bell jar which has been filled with vapor of ammonia, the white residue will gradually become dark-red. If this experiment be performed in the laboratory where there is considerable ammonia in the atmosphere, the red color will frequently appear as soon as the chlorine water and acid have been evaporated. Hypoxanthin gives this same test, and a similar one, known as the murexid test, is given by uric acid.

If a hot aqueous solution of carnin be treated with a saturated solution of bromine water, gas will be given off and the brown color of the mixture will shortly disappear. If more bromine water be added until the color is permanent and the mixture be concentrated and allowed to cool, the hydrobromide of hypoxanthin will form in needle-shaped crystals. This change is represented by the following equation:



§ 131 Inosit, also known as muscle-sugar, is found not only in muscle, but also in the vegetable world, especially in green



fruits and grains. It is present in the urine in diabetes mellitus, and in some forms of albuminuria. The muscular tissue of those long accustomed to the excessive use of alcohol, contains more inosit than that of healthier persons.

*Preparation.*—(1) Inosit is best prepared from the muscles of the heart. Cut the heart of an ox into fine bits; put these into a beaker; cover with water and stir occasionally for four hours; then filter through a cloth, pressing the residue; stir the residue with more water in a beaker, and again filter through a cloth; slightly acidify these united extracts with acetic acid, boil and remove the coagulated albumen by filtration. Concentrate the filtrate; add a solution of normal acetate of lead, and remove the precipitated chlorides, phosphates, sulphates and carbonates by filtration. To this filtrate, freed from excess of inorganic acids, add some basic acetate of lead which throws down a precipitate containing impure inosit. Collect this precipitate, wash it with water, then suspend it in water and treat the mixture with a current of hydrosulphuric acid gas. Remove the precipitated lead sulphide by filtration; concentrate the filtrate to a small volume; decant from any crystals that may form; add alcohol to the clear fluid, and set aside when inosit will crystallize.

(2) Inosit may be obtained also by the method of Boedeker, which is as follows: To the syrup from which crystals of kreatin have been obtained (see preparation of kreatin), add from one to four times its volume of boiling alcohol. If a sticky, pasty precipitate forms, decant the supernatant clear fluid; but if a flocculent precipitate is formed, filter the solution through a warm filter. The clear fluid which has been decanted or the filtrate, after standing 24 hours, deposits crystals of inosit. The pasty precipitate, if such has formed, contains some inosit; consequently such a precipitate is dissolved in a little hot water, and this solution is treated with four times its volume of boiling alcohol and the supernatant fluid decanted from any residue and allowed to stand for 24 hours when the inosit will be deposited.

If the alcoholic solution fails to deposit inosit after stand-

ing 24 hours, add to it ether until a cloudiness appears and remains on agitating the fluid; then allow to stand for 24 hours longer, when inosit will be deposited in glistening scales.

*Properties.*—Pure inosit forms in large rhombic plates and prisms, and contains two molecules of water of crystallization. It is soluble in water, insoluble in cold alcohol and ether. Its aqueous solution has a sweet taste, dissolves but does not reduce cupric oxide, does not undergo any kind of fermentation with yeast, and has no effect upon polarized light. By long exposure to the air at ordinary temperature or more rapidly at  $100^{\circ}$ , the crystals lose their water of crystallization and become opaque. When heated, inosit melts at  $210^{\circ}$ , and after cooling forms in fine needle-shaped crystals.

Inosit boiled with Fehling's solution does not reduce the copper, but changes the color of the solution from blue to green. It does not produce a brown coloration when boiled with potassium hydrate, or, in other words, fails to give Moore's test for sugar. It will be seen that inosit resembles grape sugar in its chemical composition, but the failure of the former to respond to the ordinary tests for the latter affords an easy method of distinguishing between the two.

If inosit be dissolved in water containing albumen and the solution be set aside in a warm place, as the albumen decomposes the inosit will be broken up, forming lactic and butyric acids. If an aqueous solution of inosit be boiled with basic acetate of lead, a jelly-like mass is precipitated.

Inosit is not changed by being boiled with dilute hydrochloric or sulphuric acids. If inosit be dried at  $100^{\circ}$ , then pulverized and dissolved with stirring in cold strong nitric acid and strong sulphuric acid be added to this solution, a white precipitate is thrown down. This precipitate which is represented by the formula,  $C_6H_6O_6(NO_2)_6$ , is *hexanitroinosit* and may be dissolved in boiling alcohol from which it crystallizes on cooling in rhombic tables and prisms. After the above compound has been deposited, the supernatant clear alcohol contains another substance which it deposits in groups of needles on concentration. This is *trinitroinosit*, and has the formula,  $C_6H_6O_6(NO_2)_3$ . Both of these compounds are explosive.

GLYCOGEN,  $-C_6H_{10}O_6$ .

§ 132. Glycogen exists in the muscle, white corpuscles, and in all developing cells of the animal. The muscular tissue of the foetus is especially rich in this constituent. It has been found in the placenta in large quantities; it exists in the embryo of the chick, and is abundant in the *ostrea edulis* and *cardium edule*. During foetal life the liver contains but little glycogen, while in the adult this organ seems to be the great manufactory and store-house of this substance. Only in structural disease of the organ, or after prolonged starvation, is the liver of any vertebrate animal free from glycogen.

*Preparation from the Liver.*—Kill a large rabbit, in full digestion, by decapitation, quickly open the abdomen, remove the liver, cut into fine pieces and place these in a dish of boiling water. Let the pieces cook until they harden; then decant the fluid into a beaker; rub the pieces of liver up in a mortar; return the pulp to the dish; add distilled water and boil for half an hour; filter and cool the filtrate by surrounding the vessel with snow or by placing it in ice water. To the cooled filtrate add hydrochloric acid and potassio-mercuric iodide (prepared by dissolving mercuric iodide in a boiling solution of potassic iodide to saturation) alternately as long as a precipitate forms. Agitate well, allow to stand for five minutes, and remove the albuminous matters, which have been precipitated by the hydrochloric acid and potassio-mercuric iodide, by filtration. To the filtrate add alcohol, constantly stirring, until an abundant precipitate of glycogen begins to fall. An excess of alcohol is to be avoided, for after the complete precipitation of the glycogen the continued addition of alcohol will throw down other substances. Allow the precipitated glycogen to subside; then collect it upon a small filter and wash with alcohol of 60 per cent. until the filtrate is no longer rendered turbid by the addition of a dilute solution of potassium hydrate containing a little ammonia; then wash with alcohol of 95 per cent.; then with ether, and finally with more alcohol. Dry in a dessicator over sulphuric acid. The repeated washing of the glycogen with alcohol, leaves it as a fine powder which can be easily shaken from the filter.

*Preparation from Muscle.*—Kill a rabbit by puncturing the medulla oblongata, open the abdominal walls, insert a canula into the abdominal aorta and inject as quickly as possible a solution containing one per cent. each of sodium chloride and carbolic acid. Continue the injection until the fluid returning through the inferior vena cava is colorless. This usually requires from one-half to three-fourths of an hour. Cut the muscles of the thigh into small pieces, throw them into boiling water and proceed to extract the glycogen as directed above from the liver. Muscle treated in this way yields from .03 to .35 per cent. of glycogen.

*Properties.*—Glycogen is a white, amorphous, tasteless, odorless powder, which is freely soluble in water, insoluble in alcohol and ether. If it be dried without having been previously washed with strong alcohol, it forms a pasty mass. The aqueous solution of glycogen is opalescent, but becomes clear on the addition of potassium or sodium hydrate. The aqueous solution is dextrorotatory, turning the light three times as far as a similar solution of grape sugar. On concentrating an aqueous solution of glycogen, a pellicle forms on the surface of the liquid. Filtration through animal charcoal removes the whole or the greater part of the glycogen from its solution.

If freshly prepared glycogen be treated with a solution of iodine (sufficient metallic iodine dissolved in a solution of potassium iodide to impart a wine-red color to the solution), the glycogen is stained red; if dried glycogen be treated in the same manner, a brown color is produced. If glycogen be boiled with dilute hydrochloric acid, the former is converted into grape sugar; the same change is produced by the action of the saliva, pancreatic juice or blood. It dissolves, but does not reduce cupric oxide. In an ammoniacal solution of copper sulphate, glycogen dissolves, forming a deep blue solution from which it is precipitated on the addition of nitric acid. By the action of cold, strong nitric acid, it is converted into xyloidin; on being boiled with dilute nitric acid, oxalic acid is produced. By prolonged boiling with strong alkalis, glycogen is decomposed. The addition of lead acetate to an aqueous solution of glycogen,

simply produces a turbidity, and, if this solution be treated with a current of hydric sulphide, the lead sulphide remains suspended until an alkali is added.

If to an aqueous solution of glycogen a few drops of blood be added and the mixture be kept on the water-bath for some time at a temperature of  $40^{\circ}$ , then freed from albumen and tested with Fehling's solution, sugar will be found to be present. The blood acts as a ferment converting the glycogen into sugar: this conversion consisting in the assumption of a molecule of water. A similar test should be made with a mixture of saliva and an aqueous solution of glycogen.

It will be seen both from the formula and from its various reactions that glycogen is a starch. It is especially abundant in the liver of animals which have been fed upon starchy or saccharine food. In some animals, the rabbit, for instance, after prolonged fasting the glycogen entirely disappears from the liver. Food consisting principally of fat does not increase the amount of this substance. What becomes of the glycogen of the liver is a question not positively decided. It is supposed to be gradually converted into sugar which is oxidized in the blood and assists in the production of muscular activity; but how the blood oxidizes the sugar is not known.

#### PARALACTIC ACID,— $C_8H_8O_8$ .

§ 133. This substance, known also as sarcolactic acid, is formed when a muscle contracts, and when rigor mortis sets in. It has already been stated that the reaction of living muscle, when at rest, is neutral or alkaline; and by causing contractions, the reaction becomes acid. This change is due to the development of paralactic acid.

Another form of lactic acid, (ethylidene lactic acid) has been found in the bile, in the urine after poisoning with phosphorus and in the bones in cases of osteomalacia.

*Preparation.*—This acid, which was first obtained by Wislicenus, is prepared as follows: To Liebig's extract of meat add four times its volume of tepid water; to this add, constantly stirring, about 8 parts of alcohol which throws down a precipitate; allow this precipitate to subside and decant the supernatant

fluid. The greater part of the paralactic acid is contained in the fluid which has been decanted, but traces remain in the precipitate. In order to remove these traces, stir up the precipitated matters with warm water, add alcohol, allow to stand and again decant. Unite and concentrate the alcoholic solutions to a syrup on the water-bath; extract with alcohol; again evaporate the alcoholic solution to a syrup on the water-bath; render this syrup acid by the addition of a small quantity of sulphuric acid; then shake well with ether; remove the ether and agitate repeatedly with this agent. Evaporate the united ethereal extracts, when impure paralactic acid remains; dissolve this residue in a little water, add some lead carbonate, boil and filter; treat the filtrate with a current of hydrosulphuric acid gas and again filter. Boil this filtrate until all the odor of hydric sulphide disappears and to this solution while yet hot, add zinc carbonate to neutralization. Zinc paralactate is formed and remains in solution. Concentrate the solution until on cooling crystals begin to form; then add five volumes of alcohol of 90 per cent. After standing a while, the mixture becomes turbid and is then filled with minute crystals. These may be collected upon a filter and washed with alcohol; they may be purified by repeated solution in water and precipitation with alcohol.

The crystals, as prepared above, are composed of zinc paralactate and the free acid may be obtained by treating a cold saturated aqueous solution of the crystals with a current of hydric sulphide gas, removing the precipitated zinc sulphide by filtration, concentrating the filtrate to a syrup, extracting this syrup with pure ether, filtering the ethereal solution and allowing to stand until the ether evaporates spontaneously, when paralactic acid will remain as a syrup.

*Properties.*—Paralactic acid is, at ordinary temperature, a liquid of a syrupy consistency and miscible with water in all proportions. It combines with many bases, acting as a mono-basic acid and forming characteristic compounds. Of these, one of the most important is zinc paralactate, which by the spontaneous concentration of its aqueous solution forms in fine prisms often arranged in bundles. Calcium paralactate is formed when cal-

cium hydrate is boiled with paralactic acid, the excess of calcium removed by precipitation with carbonic acid gas and filtration and the filtrate concentrated.

### NERVOUS TISSUE.

§ 134. A complete analysis of the brain or nerves has never yet been made. The substances composing this tissue are of a very complex organization, are separated from one another with great difficulty and at best but imperfectly, and some of them are probably more or less changed during the process of extraction. A long list of chemical substances obtained from the brain has recently been given, but such a list must be accepted with caution; for a great many of the ultimate analyses from which the formulæ of these substances are computed have most likely been made upon mixtures rather than pure chemical compounds. Consequently a full history of all the substances which some claim to have discovered in the brain will not be given here; only a few of those best known and most thoroughly studied will be noticed.

### CEREBRIN.

§ 135. The formula of this substance is probably  $C_{17}H_{33}NO_3$ . It was first prepared by Müller who made many analyses of it and deduced the formula given above. Otto discovered a substance resembling Müller's cerebrin but containing no nitrogen.

*Preparation.*—Free a brain from its membranes and blood-vessels as completely as possible; wash with cold water; rub the brain up in a mortar; cover the pulp with cold dilute alcohol and allow to stand for three days with frequent stirring; then decant the alcohol. The alcoholic extract contains lecithin and neurin and may be used for the preparation of these, but it contains no cerebrin. The residue of brain insoluble in alcohol is now repeatedly extracted with ether as long as this reagent dissolves any thing, ascertained by allowing a few drops of the ethereal extract to evaporate spontaneously and observing whether any residue be left or not. The ether dissolves cholesterolin and lecithin but not the cerebrin. The residue which has proven to be insoluble in cold alcohol and ether is now boiled with alcohol with frequent stirring and the mixture while

yet hot is filtered. The residue upon the filter is repeatedly washed with boiling alcohol. The united filtrate and washings are allowed to cool, when cerebrin mixed with lecithin is deposited. The cold supernatant alcohol is removed by either filtration or decantation; the residue consisting of impure cerebrin is repeatedly washed with cold ether in order to remove the lecithin, then boiled for an hour with barium hydrate. This mixture is then treated with a current of carbonic acid gas which precipitates barium carbonate and with it the cerebrin; filter and wash the precipitate first with cold water then with cold alcohol; suspend the precipitate in alcohol, boil and filter while hot. The boiling alcohol has extracted the cerebrin from the barium carbonate and as the alcoholic filtrate cools, cerebrin is deposited. For further purification, the cerebrin is redissolved in boiling alcohol, from which it is deposited on cooling then finally washed well with ether and dried over sulphuric acid.

*Properties.*—Prepared as above, cerebrin forms a white, odorless, tasteless, hygroscopic powder which consists of microscopic granules. It is insoluble in cold water, alcohol and ether, soluble in boiling alcohol or ether. In boiling water, it forms a pasty mass and dissolves to a slight extent; it is insoluble in boiling alkalis. When boiled with dilute mineral acids, cerebrin is quickly decomposed forming a sugar-like substance with lævoptatory power, but incapable of undergoing alcoholic fermentation, and another substance whose properties have not yet been studied. Cerebrin is decomposed only after prolonged boiling with an alcoholic solution of potassium hydrate. With concentrated sulphuric acid, cerebrin is converted into an oily mass which at first is of a beautiful purple color, then gradually becomes brown and finally black.

Moist cerebrin, especially when mixed with lecithin, appears under the microscope as granules or more frequently as fibres more or less twisted. Solutions of cerebrin in hot alcohol are without action upon litmus paper. If some cerebrin be placed upon platinum foil and gradually heated, it becomes brown at 80°, then melts and finally burns with a reddish flame.



LECITHIN,  $\text{—C}_{42}\text{H}_{84}\text{NPO}_9$ .

§ 136. Lecithin is found in both the vegetable and animal, as a constituent of the fluids of the cell in the former and in all the principal fluids of the latter. It is a constituent of spermatic fluid, of the fluids and yolk of the egg, of the blood, bile, transudates, nerves and brain. It may be prepared from any of the above mentioned substances, but is generally obtained from either the brain or the yolk of the egg, since these are rich in lecithin.

*From Egg-Yolk.*—(1) Hoppe-Seyler prepares lecithin from the yolk of eggs as follows: The yolks freed from the whites are shaken with successive portions of ether, as long as any decidedly yellow tint is imparted to the ether. The removed ethereal extracts are discarded and the residue remaining insoluble in ether, is treated with a large excess of water, filtered, pressed and then extracted with alcohol on the water-bath at a temperature from  $50^{\circ}$  to  $60^{\circ}$ . The alcoholic extract is concentrated to a syrup as quickly as possible at the above temperature. This syrup is dissolved in a little absolute alcohol, and the filtered solution is kept in a covered glass vessel for from 12 to 24 hours at a temperature of from  $-5^{\circ}$  to  $-20^{\circ}$ . At the expiration of this time, a deposit which generally consists of granules, though sometimes of crystalline plates, forms. This precipitate is collected in the cold, pressed and dried in vacuo over sulphuric acid. By this method, its author claims that lecithin quite pure is obtained; but the loss is very great.

(2) Strecker has introduced the following method of obtaining lecithin from the yolks of eggs: Extract the yolks with a mixture of alcohol and ether; heat the extract gently until the greater part of the ether is given off, then to the remainder after cooling add a solution of platinum chloride acidified with hydrochloric acid. This precipitate is a double salt of lecithin and platinum, is soluble in ether and is precipitated from its ethereal solution on the addition of alcohol; consequently, it is purified by being repeatedly dissolved in ether and precipitated with alcohol. Finally the ethereal solution is treated with a current of  $\text{H}_2\text{S}$  gas and the precipitated platinum sulphide

removed by filtration. The filtrate containing the lecithin is evaporated at a gentle heat. According to Hoppe-Seyler, lecithin prepared by this method is by no means pure. Evidently the lecithin thus obtained contains chlorine which may be removed by boiling an ethereal solution of the impure lecithin with silver oxide, removing the precipitated silver chloride by filtration and the excess of silver from the filtrate with  $H_2S$  gas and a second filtration.

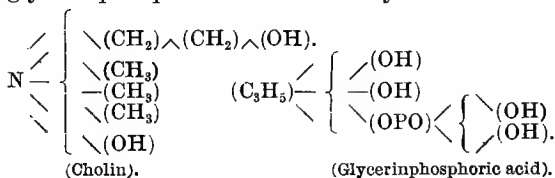
Instead of the chloride of platinum the same salt of cadmium may be used to precipitate the lecithin. In this case the double salt of lecithin and cadmium may be washed with ether, in which it is but sparingly soluble, and be dissolved in alcohol acidified with hydrochloric acid. The use of cadmium chloride has the advantage that the precipitate may be freed from fat by ether.

*From the Brain.*—A brain freed from its membranes and blood-vessels is rubbed up with a little water; the pulp kept at  $0^\circ$  is repeatedly extracted with ether; the residue is freed from any water or ether by pressure; the cake is digested with alcohol at a temperature of  $40^\circ$ ; the mixture is filtered while warm; the filtrate is kept at or below  $0^\circ$  for some time, when impure lecithin containing cholesterin is deposited; this is collected upon a filter and washed with cold absolute alcohol and ether. The mass is again dissolved in alcohol at  $40^\circ$  and the solution is surrounded by a freezing mixture, when lecithin is in part deposited while another part remains in the solution and is obtained by evaporation.

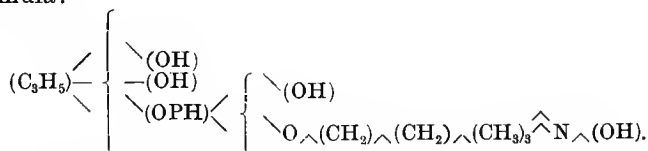
*Properties.*—Lecithin prepared by the method of Hoppe-Seyler is a brittle, colorless substance which is soluble in alcohol, very freely soluble in hot alcohol, less but yet quite soluble in ether, also soluble in benzole, chloroform and carbon bisulphide. In hot water, it swells and forms a pasty mass but does not dissolve.

If lecithin be boiled with barium hydrate, it is soon decomposed with the formation of cholin or neurin, glycerinphosphoric acid and fatty acids; of these the last two combine with the barium. On the other hand if an ethereal solution of lecithin

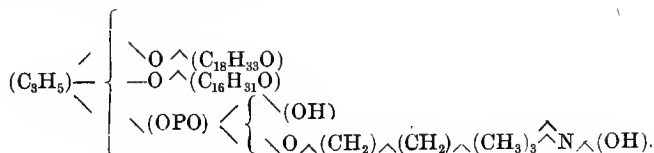
thin be shaken with dilute sulphuric acid, the acid takes up the cholin, while *distearylglycerinphosphoric acid* remains in solution. If the ethereal solution be decanted and the sulphuric acid be precipitated by barium hydrate and the excess of barium removed by carbonic acid gas and the filtrate be evaporated, cholin will be obtained. (Hoppe-Seyler). It will be well to consider the construction of lecithin; for in this way only a correct idea of the complex composition of this highly organized substance can be obtained. It is formed by the combination of cholin, glycerinphosphoric acid and fatty acids.



If now cholin and glycerinphosphoric acid unite, water will be formed taking one molecule of hydroxyl from the cholin and one atom of hydrogen from the glycerinphosphoric acid. Such a compound would be represented by the following formula:



If now one molecule of oleic acid,  $\text{HO}(\text{C}_{18}\text{H}_{33}\text{O})$ , and a molecule of palmitic acid,  $\text{HO}(\text{C}_{16}\text{H}_{31}\text{O})$ , unite with the above compound, two molecules of water and one of oleopalmitic lecithin would be formed and the latter may be represented by the following formula:



If stearic acid enters into the above combination instead of oleic acid and palmitic acid, stearin-lecithin would be formed

and from what has already been given, the student will be able to write its rational formula.

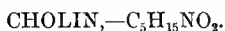
GLYCERINPHOSPHORIC ACID,— $C_3H_5PO_6$ .

§ 137. This acid is found in the body only as it results from the decomposition of lecithin; it is found in the brain in cases of softening of that organ, in the blood and urine in leucocythæmia, and in various transudates. It can be prepared from the yolks of eggs, from brain or from any substance containing lecithin. It may also be prepared by the direct action of glacial phosphoric acid upon glycerin. It is a syrupy fluid which at ordinary temperature slowly breaks up into glycerin and phosphoric acid. It is a dibasic acid forming salts with various bases; of these, the barium and calcium compounds are insoluble in absolute alcohol, soluble in water. The calcium salt is less soluble in hot than in cold water and it crystallizes from its solution in the latter on being raised to the boiling point.

*Preparation.*—Mix pulverized glacial phosphoric acid and glycerin kept at low temperature; solution accompanied by considerable increase of temperature takes place and glycerinphosphoric acid is formed. Dilute the solution with water and neutralize with barium carbonate in order to remove any excess of phosphoric acid. Remove the precipitated barium phosphate by filtration and add to the filtrate a few drops of dilute sulphuric acid in order to precipitate any barium and again filter; concentrate this filtrate in vacuo over sulphuric acid; it will be impossible to obtain the glycerinphosphoric acid perfectly free from water for if the temperature be raised sufficiently to drive off the water, the acid will be decomposed.

*Detection and Estimation.*—For the detection and estimation of glycerinphosphoric acid in animal fluids or in the brain, the following process may be used: Rub the brain up in a mortar with an excess of barium hydrate; or render the fluid, as the blood or urine, alkaline by the addition of barium hydrate; heat gently in order to coagulate albuminous matters; filter; remove the excess of barium from the filtrate by treatment with carbonic acid and filtration; concentrate this filtrate to a small

volume on the water-bath; allow to stand for some hours; pour off the fluid from any crystals of kreatin which may have been deposited; concentrate this fluid in vacuo over sulphuric acid; extract with absolute alcohol which removes urea and other substances soluble in this menstruum; dissolve the residue, which has proven insoluble in absolute alcohol, in a little water; filter; evaporate the filtrate to dryness; rub this residue up with some powdered sodium carbonate and potassium nitrate and keep the mixture at a red heat in a porcelain or platinum crucible until all the organic matter is destroyed; dissolve the cooled ash in a little water; to this solution kept at about  $40^{\circ}$ , add a nitric acid solution of ammonium molybdate; allow to stand for 24 hours, then collect upon a filter the yellowish-white precipitate of ammonium phosphomolybdate which has formed if glycerinphosphoric acid were originally present; dissolve this precipitate in dilute ammonium hydrate; to the clear solution add ammonium chloride, ammonium hydrate and magnesium sulphate. This throws down ammonio-magnesian phosphate which may be collected, dried, heated and weighed as magnesium pyrophosphate,  $Mg_2P_2O_7$ . (See p. 31). From this the amount of phosphorus, of glycerinphosphoric acid and of lecithin may be computed.



§ 138. Cholin, also known as neurin, exists normally in the body as a constituent of lecithin and when free is due to decomposition of lecithin.

*Preparation.*—Shake the yolks of eggs freed from the whites, first with ether, then with warm alcohol; remove the ether and alcohol from the united extracts by distillation; boil the residue for an hour with barium hydrate in order to decompose the lecithin; treat the mixture with a stream of carbonic acid gas which precipitates all the barium not combined with the glycerinphosphoric acid; remove the precipitated barium carbonate by filtration; concentrate the filtrate at a gentle heat on the water-bath to a syrup; extract the syrup with absolute alcohol which dissolves the cholin, but does not dissolve the barium salt of glycerinphosphoric acid; to the filtered alco-

holic extract, acidified with hydrochloric acid, add a solution of platinum chloride. The double chloride of platinum and cholin, which is formed, is insoluble in absolute alcohol and falls as a bright-yellow precipitate. Collect this precipitate upon a filter; wash with absolute alcohol; dissolve in water; treat the aqueous solution with  $H_2S$  gas and remove the precipitated platinum sulphide by filtration; concentrate the filtrate to a syrup on the water-bath and dry in vacuo over sulphuric acid. In this way the chloride of cholin is formed and may be freed from chlorine by dissolving in water, shaking with recently precipitated silver oxide, and filtering.

*Properties.*—Cholin is a colorless syrup of a decidedly alkaline reaction, soluble in water and alcohol and unites with acids forming salts which are easily decomposed. The most characteristic of its salts are its double chlorides with platinum and gold. The former is soluble in water, insoluble in alcohol and ether and is deposited from its concentrated aqueous solution, after standing over sulphuric acid, in large orange-colored rhombic prisms or six-sided plates, having the composition represented by the formula,  $(C_5H_{15}NOCl) Pt Cl$ . The double chloride of cholin and gold forms in fine yellow needles, which are also insoluble in alcohol and ether, and which become brown on being heated. The chloride of cholin forms in colorless prisms, needles or plates, the latter often resembling the corresponding crystals of cholesterin. This salt is soluble in alcohol, but insoluble in ether.

## THE URINE.

### COLOR.

§ 139. The normal urine of man is of a golden yellow color; while from various causes, some transient and unimportant, others more permanent and serious, this excretion may so vary in appearance as to present almost every shade of color. It must be remembered that what will be here given concerning the color of the urine applies only to the fluid and not to any deposits; consequently should any deposit be present, the same should be removed by filtration and the color of the clear filtrate determined. The color of the urine

may be regarded as depending upon these two conditions, (1) variations in the proportion of normal coloring matters present, (2) the introduction of abnormal coloring matters.

Pale urine is the result of an excess of water in this excretion and may be colorless. It may be alkaline, neutral, or feebly acid, and is the normal urine of infancy and of extreme old age; while in others it may be due to the consumption of a large quantity of water either as such or as contained in food, especially vegetables and fruits, or to a pathological condition of the system as in diabetes, chlorosis, anæmia and hysteria. Pale urine is generally of low specific gravity, the urine of diabetes mellitus being an exception to this rule. In all pale urines, the normal coloring matters are deficient in proportion to the water and the color of such urine is heightened by concentration.

On the other hand, if the normal coloring matters be in excess in proportion to the water, the urine will be more or less highly colored. This is the case when but little water is taken or when the water leaves the body through other avenues than the kidneys; thus, the urine excreted when the perspiration is greatly augmented is small in quantity, strongly acid and highly colored. Concentration has taken place in the body producing the same result as if the normal quantity of urine had been passed and then concentrated by the application of heat. Again the urine will be highly colored when it contains an excess of nitrogenous constituents. This may result from the consumption of much nitrogenous food or from the rapid disintegration of tissue as the result of disease; from the former cause the urine of the carnivora and of man, when living principally upon nitrogenous food, is highly colored, while from the latter cause result the reddish urines of febrile diseases. One of the sources of the normal coloring principles of the urine is in the process of the retrograde metamorphosis of muscular tissue, and in this respect the same result follows, whether it be from the disintegration of the muscle of the ox taken into our bodies as food or by similar changes going on in our own muscular system as the result of disease.

The abnormal coloring matters of the urine may be divided into two classes: (1) those which result from food or medicines; (2) those which are due to pathological conditions of the body. In some persons the coloring principle of coffee is soon excreted by the kidneys and gives to the urine a brownish tint. Rhubarb, senna, santonin, hæmatoxylon, carbolic acid, creosote, tar, and many other medicinal agents influence the color of the urine. Rhubarb colors the urine a greenish-brown, and often leads one to suspect the presence of bile-pigments. If a dose of santonin be taken and the urine for the next 24 hours be collected it will appear normal in color if it be acid, but upon the addition of an alkali the urine will become crimson. It must be remembered that the addition of santonin and an alkali to normal urine will not produce this color; it is due to the action of the alkali upon the substance into which the santonin is changed during its passage through the body. Either the internal or external use of carbolic acid or creosote will often cause the urine to be more or less dark, sometimes quite black; an inunction of tar will produce the same result.

*Colors of the Urine Produced by Pathological Conditions.*—Greenish-brown or reddish-brown urine may result from the presence of bile-pigments. Blood may produce a variety of shades; thus if the bleeding be from the bladder or urethra, and especially if it be profuse the coloring matter yet existing as hæmoglobin, the color will be red; while if the blood has passed through the kidney, the corpuscles will often be disintegrated, and the coloring matter so changed as to give to the urine a smoky or dark tint, and indeed it may be black. In some rare cases, the urine after standing for some time becomes blue or more frequently a blue pellicle forms upon the surface or blue granules are deposited. This has been observed in various forms of albuminuria and in diabetes mellitus and is due to the oxidation of indigo-forming substances.

§ 140. *Significance of the Color.*—The fact that a specimen of urine is of a normal color is not proof sufficient that it is normal in other respects. The pale urines indicate either a tem-



porary excess of water or some chronic disease, never an acute form; while the highly colored, the red, brown and dark varieties are indicative of acute forms of disease, unless they be produced by the food.

#### THE AMOUNT OF URINE.

§ 141. Formerly it was thought that it was only necessary to estimate the per cent. of urea and other constituents of the urine. Consequently, in many of the older works, we find long lists of figures given showing the number of parts per thousand of chlorides, phosphates, etc. A moment's thought will convince us that the great majority of these analyses are of no value. Suppose that one eats much solid food and drinks but little water and other liquids; while another eats but little solid food and consumes large quantities of some drink; is it reasonable to suppose that the number of grains of urea in a liter of the urine of each will, by any means, be the same? The old method has passed away and we now estimate the amount of urine and its various constituents passed in a given time. The most suitable period to take as the basis of our estimations is twenty-four hours; because, during this time man passes through a cycle of changes, which with greater or less variations are repeated every subsequent day.

Having decided upon the time for which the urine should be collected, the next question is how should it be done. It is necessary that the vessel should be perfectly clean, and we use this word, clean, in a scientific sense and not according to the ordinary acceptance of the term. Patients, who should know better, when requested to collect their urine will often bring it to the physician in a bottle from which they had poured some oil, rinsed it with a little water and called it clean. In order to cleanse a bottle for this purpose, it is best to wash it out first with water, then with a solution of caustic potash, again with water, then with dilute sulphuric acid, and then rinse it with distilled water until the rinsings cease to give an acid reaction when tested with litmus. The patient is then instructed to completely empty his bladder at a certain time, throwing this discharge away, and to collect in the prepared vessel all the

urine excreted until the same hour of the next day. Caution must be taken to prevent loss of urine when at stool. After it has been collected, the urine should be measured in clean glass jars or cylinders graduated according to either the French or English system.

When we remember that in health, the kidney is one of the channels through which the excess of water passes from the system, we shall appreciate the fact that in a healthy condition, the amount of urine will vary (1), with the amount of water ingested and (2), with the quantity that leaves the body by other avenues. To these, we must add a third physiological condition, which is constantly influencing the quantity of the renal secretion, *i. e.*, the quality and quantity of solid food.

As a rule, the quantity of urine is from one-tenth to one-half more than the amount of water drank; but it must be borne in mind that this proportion may be reversed by excessive perspiration or by watery stools. I found, in experimenting upon this subject, that when the average mid-day temperature was 72° F., in the shade, for every 1000 c. c. of water drank, I excreted 1220 c. c. of urine. In this case, I took but little exercise. The excess of water in the renal excretion over that ingested comes partly from the water contained, as such, in the solid food and partly from the oxidation of the hydrogen of the food. Moreover, when the atmosphere is very damp, more water may be absorbed through the lungs than is exhaled. It will be seen from this that by increasing or decreasing the quantity of water drank, we can, as a rule, correspondingly increase or decrease the amount of urine excreted in a given time. Can we make any use of this fact in treatment? We can in case the daily excretion of urine is too small, but if, on the other hand, it is too large, I doubt the propriety of restricting the patient in the gratification of his thirst. In all such cases as the latter, the cause of the trouble lies deeper than the mere consumption of an excess of water, and this cause must be sought for, and the treatment directed to it; because the abnormal thirst is but an effect and follows the cause just as necessarily as darkness follows the withdrawal of light. I knew

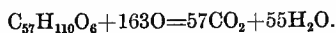
of a case of diabetes insipidus, which a man who wrote M. D. after his name (we suppose that in this case, these letters signify *disgrace to medicine*), treated by locking his patient in a room and allowing her but a small quantity of water. The thirst still existed and its gratification was a necessity; consequently, the urine, as soon as it passed, was swallowed by the patient in vain endeavor to relieve her unbearable suffering. These diseases in which there is an excess of urine passed, will be discussed in subsequent chapters. Fortunately for the physician the majority of his cases in practice will belong to the former class, *i. e.*, when there is a deficiency of the urinary excretion. I want to impress the importance of attending to this subject; because it has been overlooked by too many. A great many persons drink too little water. The merchant goes behind his counter and in order to avoid frequent visits to the water-closet, drinks but little water; consequently, his urine is small in amount, of high specific gravity, strongly acid, and often deposits urates, uric acid and oxalate of lime in the urinary passages. The result is irritation of the bladder with cystitis, or a stone is formed. If the physician sees him in time to avoid these disastrous consequences and advises him to drink more, the reply often is, "Give me some medicine for it; I do not want to drink much water or I will have to go out every hour." As soon as his bladder becomes irritated, micturition will necessarily be more frequent and his own actions compel him to traverse a rougher road than the one which he endeavored to shun. It must not be supposed, by my specifying the merchant that this class only commit this error. The same mistake is made by ladies who are out in society much; by the student who does not wish to be interrupted in his studies by the calls of nature; and even by the physician, who is so constantly attending to the wants of others, that he forgets his own. From the foregoing, I think that we are justified in deducing the following rule: If your patient complains of some irritation of the urinary tract and upon examination you find the amount of urine 1000 c. c. or less, the specific gravity 1028 or higher, the reaction strongly acid, no sugar or albumen, have him measure the amount of

water that he drinks during twenty-fours and see if it is not correspondingly small. If this be the case, it is well to give some mild diuretic dissolved in much water. For this purpose, citrate of acetate of potassium will often be found very suitable; because, during their passage through the body, these salts are converted into carbonates, which will decrease the acidity of the urine. It must be remembered that in no case should these remedies be used in quantities sufficient to render the urine alkaline.

We have next to consider the effect produced upon the daily amount of urine by the quantity of water excreted through other channels. One day when the mercury went up to 100° F. in the shade, I walked eleven miles at the rate of three miles an hour and spent the remainder of the twenty-four hours in my room, comparatively inactive; during this time I drank 2000 c. c. of water and excreted 562 c. c. of urine. It is owing to the diminished cutaneous exhalation, that more urine is excreted in winter than in summer. Whether normal sweat contains any urea or not is a question still under discussion. Funke and others claim to have found it present in large quantities; but it is evident that either they mistook something else for urea, or the sweat which they examined was not normal. My own opinion, founded upon experiments, is that in a perfectly healthy condition urea is not a constituent of perspiration. Be this as it may, it is well known that when the kidneys are so changed in structure as to fail in the performance of their function, not only urinary water, but the solids, both organic and inorganic may pass off through the skin. Consequently, in these diseases, the intelligent physician often causes, by means of the hot air-bath, a profuse flow of perspiration and in this way removes from the blood, urea, uric acid and other poisonous substances. In such cases, urea or the product of its decomposition, carbonate of ammonia, is also excreted by the lungs.

The third physiological factor, upon which the daily excretion of urine depends, is the solid food—its quality and quantity. It was long ago observed that man passed more urine

when living upon animal food, than when he subsisted upon vegetables. Lehmann found that when his daily rations consisted of 39.79 oz. of animal food (eggs), he excreted 1202.5 c. c. of urine; while when he ate the same amount of vegetable food, he passed 909 c. c. of urine. In a series of carefully conducted experiments, I found that when I consumed in my food daily 225 grains of nitrogen, the average amount of urine was 960 c. c.; and when my food contained 155.9 grains of nitrogen, the urine excreted amounted to only 769 c. c. In these experiments all the food was weighed and the drink measured and the only change which was made and which reduced the quantity of urine was the withdrawal of solid nitrogenous food. The effect of the kind of food has been observed in the lower animals. A cat, fed exclusively upon animal food, excretes seven and a half times as much urine for every pound of its body weight, as the horse, fed upon corn and hay, excretes. Many other experiments might be cited to show that the quantity of urine depends upon the quality of the food—whether it be animal or vegetable or mixed—and upon the quantity of nitrogen which it contains. The explanation for these facts is that nitrogenous food is a true stimulant and increases the rapidity of certain chemical changes going on in the body. Nitrogenous food hastens the oxidation and the consequent excretion of not only the non-nitrogenous substances that are taken in at the same time with the food, but also of the fat that may be stored up in the body. It will be remembered that a Mr. Banting proposed to reduce corpulent persons to any desired extent by feeding them exclusively upon animal food. His theory depends upon this fact, that the nitrogenous substances by acting upon the nerves increase the oxidation of the fat which has been stored up. The formula for stearin is  $C_{57}H_{110}O_6$ . It contains much hydrogen and when it is oxidized a corresponding amount of water will be formed as seen from the following equation:



We must free our minds of the old belief that the sole or even the principal office of nitrogenous food is to repair the

waste of the muscular system; for we have no evidence that such waste exists to any considerable extent; but it is evident that this kind of food is a true nerve stimulant. I hope, though, that none of you will employ Mr. Banting's plan of reducing corpulency. Consider the extra amount of work that is thrown upon the kidney in eliminating the great quantities of urea, to say nothing of the water. Moreover, there is a safer and more reasonable way of removing any superfluous fat, as has been pointed out when discussing foods\*.

Many other conditions have been mentioned by authors as influencing the amount of urine. As a rule women pass less urine than men; this is not due to any mysterious influence that sex has over this excretion; but depends upon the fact that women eat less and are not so constantly engaged in physical exercise. It is equally evident why children pass more and old people less urine in proportion to the body weight than those in the prime of life. There are certain articles of food and drink which have a diuretic effect. This is true of onions, tea, coffee, and wine or beer.

It will be seen from the preceding considerations, that it would be impossible to give exactly the number of cubic centimeters that constitute the normal daily excretion. An amount, which under certain conditions would indicate a serious disorder, would under other circumstances be a result of healthy action. In the examination of urine for either physiological or diagnostic purposes, the physician must be, as he should be in all of his professional work, both broad and deep in his observations. Every day I see something which impresses upon me the belief that the most thorough analyses of the egesta are of but little value, as aids to treatment, without a corresponding knowledge of the ingesta and of the conditions surrounding the patient. There are these three important factors, (1) the quality and quantity of the ingesta, (2) the atmosphere in which the patient lives, and (3) the quality and quantity of the egesta, that should always be inquired into by the physician. There is now a tendency among medical men to

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\* The Physician and Surgeon, July, 1879.

depend too much upon the detection of abnormal constituents of the excretions and to neglect other investigations. For instance, a patient complains of nervousness, indigestion, and probably of some slight irritation of the urinary passages, the urine is examined and found to contain large quantities of calcium oxalate and uric acid, the physician inquires no farther, and prescribes nitro-muriatic acid. The prescription is all right, but the patient may be eating, all the while, such large quantities of starchy food, that the most heroic doses of nitro-muriatic acid will not suffice to oxidize it all; or he may be sleeping every night in a room so poorly ventilated that the amount of oxygen inhaled is only sufficient to convert the carbonaceous part of the food into oxalic acid and not enough to produce carbonic acid; or he may be drinking so much wine that uric acid is necessarily produced in excess. The study of the excretions has richly repaid its investigators, and it promises to yield to those who will continue to labor in its mines, gems brighter than any that have yet been brought to light. But we must remember that golden images cannot be cast from molten lead; nor can Alpine plants grow in the burning sands of Sahara; neither can the excretions be normal so long as the food is abnormal; nor can man enjoy health so long as he violates the laws of hygiene.

*Average for 24 Hours.*—From what has been said, we will be able to appreciate the fact that very different figures are given by different authors to represent the average daily excretion of urine. Valentin gives his average amount at 1447 c. c.; Lehmann, his at 1057 c. c.; Thudichum gives 1950 c. c. as an average for seventy-six days for a man aged 28 years, weight 70 kilos. My average, age being 26 years, and weight 65 kilos., for 100 days is 960 c. c.

*Hourly Variations.*—A study of the hourly variations in the amount of urine excreted, presents some very interesting points and enables us to understand more fully the daily cycle of changes through which man passes. I will give three tables representing the hourly excretion for three consecutive days. The day, as here understood, begins at 12 M. At the expiration

of each hour, with the exception of the time during which I slept, the urine was passed into a graduated glass and the amount noted. The figures represent so many c. c.:

TABLE NUMBER ONE.

Dinner at 12; Supper at 6; Breakfast at 9.45; Sleep from 11 P. M. to 7 A. M.

P. M.													A. M.					
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount...	50	62	65	50	35	28	27	34	39	15	19	14	189	44	52½	60	52½	52½

TABLE NUMBER TWO.

Dinner at 4.30; No supper; Breakfast at 8.30; Sleep from 11 P. M. to 7 A. M.

P. M.													A. M.					
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount	52½	52	63¾	47	37	33¼	24½	22½	19½	25	24	15	131	33¾	36½	43	18¾	45

TABLE NUMBER THREE.

Dinner at 1; no supper; no breakfast; drank 8 ozs. of water at 6 P. M. ; sleep from 11 P. M. to 7 A. M.

P. M.													A. M.					
Hour.....	12	1	2	3	4	5	6	7	8	9	10	11	7	8	9	10	11	12
Amount	45	40	24	27	16	16	12	16	17	11½	11	10	68¾	25	35	39	22½	45

In no case, with the exception indicated in table No. 3, was any food or drink taken between meals. It is very evident from the tables that from about 2 P. M. there is a gradual decrease in the amount until the hour of retiring; while on the other hand, from about 8 A. M. there is a gradual increase until mid-day. This decrease during the afternoon and increase during the forenoon is quite independent of the food and drink. Thus, in table No. 3, although dinner was taken at 1 P. M. and 8 ozs. of water consumed at 6 P. M. and neither food nor drink taken during the morning, still the forenoon increase and the afternoon decrease appear. In both tables 2 and 3, it will be noticed that the amount passed at 11 A. M. is small. This seems to be an exception to the forenoon increase; but the decrease in the amount passed at this hour is due to the fact that on each of these days, the preceding hour (from 9 A. M. to 10 A. M.) was devoted to physical exercise (walking) which caused the perspiration to flow freely. In noting this hourly variation, we



have only written another line in that great volume of facts which demonstrate the plant-like life of man. Only under the influence of sunlight is the carbonic acid decomposed and the carbon transformed into plant tissue; likewise, the light of day is essential to the full activity of the organs of digestion, absorption and excretion.

*Effects of Medicines.*—We will now briefly consider the effects of remedies upon the amount of urine. In the selection of a diuretic, the physician should first ascertain the cause of the small excretion and then treat accordingly. It would be very unwise to administer, in every case of diminished excretion of urine, acetate of potassium simply because that article is classed with the diuretics in the *Materia Medica*. Remember that rational men believe that every diseased state has its cause and in the condition now under consideration it is the cause of the diminished flow that we must endeavor to remove. The amount of urine varies directly with the arterial pressure; consequently, if there be a want of vascular fullness, water is the best diuretic that can be given. In these cases, drinking large quantities of water increases the amount of urine, diminishes its specific gravity, lessens the acidity and, consequently, soothes any irritated part of the urinary tract. In fevers, water and sweet spirits of nitre serve the double purpose of cooling the body and increasing the amount of the renal secretion, of gratifying the desire of the patient and accomplishing the object of the physician. If there be slight congestion of the kidney, as shown by the diminished excretion and by a dull pain in the loins, sweet spirits of nitre is again useful. If the urine be small in amount, strongly acid, containing free uric acid and producing irritation, acetate and citrate of potash, as has already been shown, are beneficial. But if there be general venous stasis from diseased action of the heart, digitalis should be combined with the salts of potassium. The digitalis acts upon the heart, produces free circulation, increases arterial pressure, removes the stagnating blood loaded with carbonic acid and other poisons from the kidney, and prevents those changes in the renal structure which would necessarily follow from malnutrition. The salts of pot-

ash dissolve and probably oxidize the uric acid and thus prevent the formation of gravel and calculi. Both the digitalis and potash increase the amount of urine in these cases. Brunton has shown\* that in health, this drug increases the amount of urinary water, and I have seen the daily amount of urine rise from 880 c. c. to 1100 c. c. within three days from the administration of five drops of the tincture of digitalis three times per day in a case of "irritable heart." If there be any inflammation of the urinary tract, as pyelitis, cystitis or urethritis, or if the condition known as "irritable bladder" (when the urine is concentrated and is strongly acid, and when there is a constant desire to urinate with but little relief from micturition) exists diuresis is best produced by the combination of either buchu, pareira brava, or uva ursi with a vegetable salt of potassium.† In parenchymatous inflammation of the kidney, all irritant diuretics must be either avoided altogether, or given with the greatest care.

When the flow of urine is excessive from debility and consequent relaxation, it is best to build up the system by the use of tonics. For this purpose, iron, strychnia, and quinia have proven very efficient. The treatment of diabetes insipidus and other diseases in which there is an excessive excretion of urine, will be discussed in subsequent lectures.

#### THE REACTION.

§ 142. *How Ascertained.*—The reaction of urine is best ascertained by its action upon blue and red litmus paper. If it be acid, it will color blue litmus paper red; while, if it be alkaline, it will color red litmus paper blue; and if it be neutral, it will produce no change upon either kind of the test papers. If the urine be found alkaline, it is important to decide whether this reaction is due to a volatile or to a fixed alkali. If it be due to a volatile alkali, ammonia, the blue color imparted to the test paper will disappear upon drying, but if due to a fixed alkali, the color is permanent.

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\* On Digitalis, page 43.

† H. C. Wood, *Materia Medica*, page 475.

*Reaction of the Day's Urine.*—The reaction of the mixed twenty-four hours' urine, if normal, is always decidedly acid when collected.\* This reaction is due to the presence of acid phosphate of sodium, acid urates, kryptophanic acid, probably lactic, and perhaps other organic acids. If kept in a clean vessel and in a cool place, the acidity is increased, or the urine undergoes the acid fermentation within a few days. During this process, an organic acid—probably lactic from the sugar which Pavy has shown to be present in small quantities in normal urine—is developed and unites with the bases setting free uric acid; while the latter is converted by the oxygen of the atmosphere into oxalic acid, which immediately unites with the calcium present, and the calcium oxalate thus formed is deposited in octohedral crystals. If the urine contains an excess of mucus, or if it be kept in a warm place, the acid fermentation either goes on so rapidly that it is not observed, or it does not occur at all. Be this as it may, the urine will sooner or later become alkaline. This depends upon the fact that the urea takes up two parts of water and is converted into ammonium carbonate, as represented by the following equation:



That this decomposition is hastened by the presence of mucus may be proved by pouring into one beaker any amount of normal urine *without* filtration, and into another beaker an equal amount of *filtered* urine from the same specimen; setting the two beakers away and testing the reaction of each from day to day. It will be found that the specimen which has been deprived of its mucus by filtration retains its acid reaction much longer than the other. The same fact can be proved in another way. Divide a specimen of normal urine into two equal parts; to one of these add a quantity of mucus; set the two portions aside, and test as before. It will be found that the one containing an excess of mucus is first to become alkaline. This decomposition of urea into ammonium carbonate may take place in the urinary passages, and from the experi-

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\* This statement is true only when the urine is collected in a clean vessel and kept in a cool place. During the summer season in warm latitudes, the urine will often decompose within a very few hours after emission.

ments given above, it will be seen that this is especially liable to occur when the bladder pours out pus or an excess of mucus as is the case in cystitis. Why mucus hastens the decomposition of urea and the nature of the changes, if any, that occur in the mucus itself, are subjects which are not yet understood and which deserve careful investigation. Pasteur held that the change was due to atmospheric germs which found a nidus in the mucus, and consequently the more mucus a specimen of urine contained, the more suitable was it for the development of these germs. That this theory is entirely untenable must be admitted, when we remember that the decomposition goes on in the bladder, to which air has no access.

*Effect of Food.*—While the mixed urine for twenty-four hours is invariably acid when normal, the urine passed at different hours of the day varies in reaction, and that passed at certain hours may be neutral or even alkaline, and still be normal. Dr. Bence Jones first observed that after a meal the acidity of the urine gradually decreased for a while until often it became neutral or alkaline. Dr. Roberts repeated the experiment of Dr. Jones, and found that after breakfast the acidity was sensibly decreased within forty minutes, and continued to decrease until the expiration of the second or third hour; when the urine gradually regained its acidity. After dinner there was no perceptible change until the second hour, and the greatest alkalinity was attained during the fourth and fifth hours. Dr. Jones thought that the alkalinity of the urine during digestion was due to the withdrawal of the acid from the blood to form gastric juice, and that the greater the alkalinity of the urine, the greater the acidity of the gastric juice, and *vice versa*. Dr. Roberts admits the probability of the theory advanced by Dr. Jones, but thinks it more likely that the decrease of acid in the urine after meals is due to the excess of alkalis in the food\*.

*Daily Cycle of Variations.*—I have made a great number of experiments upon the reaction of the urine passed at different times of the day, and while I think Dr. Roberts is right in

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\* Roberts on Urinary and Renal Diseases, Third American Edition, page 48 et seq.

deciding that foods influence the reaction, I am compelled to believe that he has omitted many important circumstances upon which the reaction depends. The more I experimented, and the greater variations I made, the more fully was I convinced that the reaction of the hourly excretions of urine depends upon various and complicated factors. First as to the influence of the food. The degree to which the reaction is affected by food depends upon the time of day at which the food is taken, as well as upon the kind and amount of food. The following tables, taken from a great number representing similar experiments, will illustrate my meaning. The positive sign signifies that the urine, passed at the hour indicated, was acid; while the negative sign represents an alkaline, and the cipher a neutral condition:

TABLE NUMBER ONE.

August 24. No food or drink taken until dinner; dinner at 1; supper at 6:15. Sleep from 11 P. M. to 7 A. M.

A. M.							P. M.										
Time when passed,	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Reaction.....	+	+	0	-	-	+	+	0	-	-	+	+	+	+	+	+	+

TABLE NUMBER TWO.

August 25. Breakfast at 9:45; dinner at 4:30; no food or drink after dinner. Sleep from 11 P. M. to 6 A. M.

A. M.								P. M.										
Time when passed,	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Reaction.....	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+

TABLE NUMBER THREE.

August 26. Breakfast at 8:30; dinner at 1; no more food nor drink taken until 1 P. M. of the next day. Sleep from 11 P. M. to 5 A. M.

	A. M.								P. M.										
Time.....	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Reaction....	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+

On August 23, I took supper at 6 P. M., and as indicated in table No. 1, no food or drink was taken on the 24th, until 1 P. M.; nevertheless, the urine was neutral at 9 A. M., and alkaline at 10 and 11. What caused this alkalinity? Could it have been due to the supper of the preceding evening? It is well to remark

here that during this forenoon I took no exercise; in fact did not leave my room. On the afternoon of the 24th, the urine was neutral at 2; and alkaline at 3 and 4. This was probably due to the food taken at 1 P. M. But supper was taken at 6 P. M., and the acidity of the urine passed at the expiration of each hour until 11 P. M. was estimated and not only did the secretion remain acid, but its acidity was increased. The food taken at dinner and supper of this day was weighed and, for the two meals, was identical both in quantity and quality. Here we have a certain amount of food taken at 1 P. M. causing the urine to become alkaline within two hours, while the same amount of the same kind of food taken at 6 P. M. does not lessen the acidity of the urine within five hours. On the morning of the 25th the urine was alkaline at 7, although breakfast was not taken until 9:45. Again, as shown in table No. 2, dinner was taken at 4:30, the urine being acid and had increased in its acidity at 11 P. M.; while on the subsequent morning, as shown in table No. 3, the urine was alkaline at 7, although breakfast was not taken until 8:30. It is evident, on an inspection of the tables, that food taken towards the close of day does not influence the reaction so quickly as that taken in the forenoon. It will be observed in table No. 3, that the urine was acid at 11 A. M. and alkaline during the preceding and subsequent hours. It may possibly be that the effects of the supper of the preceding day had ceased to be manifest while the breakfast had not yet produced its effects: but I think that the acidity possessed by the excretion of this hour was due to the fact that during the hour from 9 to 10 of that morning, I walked constantly and rapidly. The conclusions which I have drawn from these experiments are (1), food, taken during the latter part of the day, undergoes very slowly those changes which are necessary before it can be excreted by the kidneys and (2), exercise increases the acids of the body and consequently the acidity of the urine.

*Effects of Exercise.*—The first conclusion is but a corroboration of the facts ascertained with regard to the hourly excretion of urine and will be again emphasized when we study the variations in the amounts of urea excreted at different times of the

day. The second conclusion would not be justifiable did it rest on the experiments given above, only; but other and, to my mind, sufficient evidence is at hand. During the Fall of 1877, I found thirty students sufficiently interested in this subject to estimate the acidity of the daily excretion of urine for from two to three weeks. During six days of the week, they attended lectures and clinics and performed laboratory work; while on the seventh day of these weeks of experimentation they took long walks through the country. The urine of the seventh day invariably contained more acid than that of any other day. The hours from 8 A. M. to 12 M. of each of the six days were passed in a poorly ventilated room listening to lectures and in physical inactivity. With but one exception out of the thirty, the urine passed upon leaving the lecture room at noon was alkaline and turbid from the precipitation of earthy phosphates. In the afternoon, the students were engaged in the chemical laboratory and consequently took more exercise. During this time the urine regained its acidity and maintained it until the following morning. At length, the time for examination drawing near, the laboratory work was discontinued, and the afternoons were devoted to close study, and the urine was constantly alkaline and turbid with phosphates. When the urine is alkaline either from food or insufficient exercise, the reaction is always due to a fixed alkali. From a long list of experiments, R. Maly\* has also reached the conclusion that the acids of the body are increased by exercise. He finds that the acid phosphates of sodium are especially augmented and mentions monosodic phosphate ( $\text{Na H}_2\text{PO}_4$ ) as one of the constituents of the blood, resulting from muscular activity. The physiological evidence here given to prove that exercise in the open air increases the acids of the body is supported by clinical experience. What is the general condition of those patients, who are troubled with the deposition of phosphates in the bladder from urine alkaline with a fixed alkali? They are, so far as my experience goes, invariably those who are debilitated by age, by disease, by poverty, by either muscular inactivity or by want of pure air, or by

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\*Chem. Centr., 1878.

both. It is true, as Roberts remarks, that an excess of fixed alkali in the urine is not so injurious as a volatile alkali. The urine with a fixed alkali is bland and the amorphous phosphates seldom form a stone, but are washed out with the urine; still they often cause some irritation, especially in old men with enlarged prostate. Moreover, urine alkaline from a fixed alkali always denotes a low state of vitality and should not be disregarded by the physician. In the treatment of these cases, two objects may be kept in view. These are (1), to relieve as speedily as possible, any irritation of the bladder and (2), to increase the vitality of the patient, and in this way to remove the cause of alkalinity. The latter is accomplished by the judicious use of tonics, by exercise and pure air. The former object is best attained by the administration of the weaker mineral acids (as carbonic and phosphoric) or of vegetable acids. In quite a number of cases of old men with urine alkaline from a fixed alkali and with irritation of the bladder causing frequent micturition, I have observed that drinking old cider rendered the urine acid and relieved the irritation very promptly. Carbonic and phosphoric acids act by combining with the excess of bases, (sodium, potassium and calcium) in the blood forming acid salts which are excreted by the kidney and influence the reaction of the urine. Benzoic acid acts in a similar way, being converted during its passage through the body into hippuric acid, which combines with the bases forming hippurates. However, benzoic acid is not so useful in these conditions as it is when the urine is alkaline with ammonia. The administration of the strong mineral acids, as nitric and hydrochloric, in order to render alkaline urine acid is in accordance with neither physiological nor chemical facts.

*Ammoniacal Urine.*—If the reaction of urine be due to ammonia, one of the following may be the cause: (1) the urine has been unduly retained in the urinary passages; (2) the bladder is not completely emptied at each micturition and some stale urine is left to decompose the normal as fast as it falls from the ureters; (3) there is some undue irritation of the urinary passages causing them to pour out pus, or an excess of mucus.



The first one of these causes will be discussed fully in the lecture on retention. Suffice it here to say that the urine may be retained in the pelvis of the kidney, or in the bladder, and that the retention may be due to calculi, stricture, paralysis, enlarged prostate, morbid growths, and foreign bodies. The treatment consists in removing the cause. With regard to the second cause given above, it is well known that if normal urine be allowed to drop, at the rate which urine passes into the bladder, into a vessel containing putrid urine and the whole be kept at the temperature of the body, the urea of the normal urine decomposes very rapidly. It frequently happens that from enlarged prostate or other partial obstruction, the bladder is not completely emptied during micturition, consequently the remaining urine becomes putrid and decomposes the normal urine as it enters the bladder. In these cases, complete evacuation of the bladder should be secured, either by the removal of the obstruction, by drawing off the urine with a catheter, or, when these are impossible, by washing out the bladder frequently. To Sir Henry Thompson belongs the credit of calling attention to the fact that "*you can not completely empty every bladder with the catheter*. When the prostate is irregular in shape and throws out protuberances into the bladder, there are sinuses or spaces between them, which retain one, two or even more drachms of urine. Again there are not unfrequently numerous small saculi in the coats of the bladder which act in the same way."\*

We will now consider how irritation of the urinary passages may lead to the production of ammoniacal urine. This irritation may be due (1) to an abnormal condition of the urine when it reaches the bladder, and (2) to the presence of some foreign body. Sometimes the urine, as excreted by the kidney, is unduly acid and irritates the mucous membrane of the passages. This causes the production of an excess of mucus, and we have already seen that urine containing much mucus becomes alkaline quicker than normal urine; consequently the urea is decomposed into ammonium carbonate while the urine is yet in the bladder. Moreover, ammoniacal urine is very irri-

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\* Diseases of the urinary organs, page 194, third edition.

tating and this change in the reaction from undue acidity to alkalinity only increases the inflammation and consequently the amount of mucus. Thus the mucus and the ammoniacal urine react upon each other, the latter increasing the irritation of the bladder and the former hastening the decomposition of urea. This condition may continue for years, and render the life of the person miserable. Foreign bodies set up a similar irritation and produce the same results.

The effects of the absorption of ammoniacal urine into the blood have been studied by MM. Gosselin and Robin.\* These experimenters first ascertained the effects on animals of subcutaneous injections of an aqueous solution of ammonium carbonate. When large amounts of this were used, there were restlessness, cries, convulsive movements, slow pulse, a fall in temperature, albuminuria and diminution of the number of blood corpuscles. When but small quantities of the aqueous solution of ammonium carbonate were used, the symptoms were slight, or none were observed.

The same investigators found that small and repeated injections of normal urine caused but slight local irritation, with a limited increase of temperature; that large quantities of normal urine were necessary to produce death, and that the only change observable at post mortem was a slight renal congestion.

A mixture of ammonium carbonate and normal urine was next used. After the injection of this mixture, severe local effects were soon manifest, the temperature rapidly increased and death quickly followed. Although large quantities of normal urine and ammonium carbonate had been required, when used separately, to cause death; still, but a small quantity of the mixture proved fatal. When putrid urine obtained from patients with cystitis was substituted for the mixture, the symptoms were much more severe and death followed more rapidly.

The conclusions of MM. Gosselin and Robin are as follows: (1) "Urine spontaneously ammoniacal acts with greater intensity than a more concentrated mixture of ammonium carbonate and normal urine." (2) "Ammoniacal urine is very poisonous

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\* Archives Générales de Médecine, May and June, 1874.

when injected subcutaneously and the intensity of its action varies with the amount of ammonia." (3) "Local lesions and fever (similar to the conditions observed in the extravasation of urine) are manifest." (4) "The pathological conditions correspond with those observed after death from urinary fever." (5) "The poisonous effects are greatly increased when air is admitted." (6) "The rapidity with which normal urine decomposes, when mixed with pus and blood and in contact with air, explains how febrile accidents occur after operations on the urinary organs, even when the urine was acid before the operation."

These valuable experiments enable us to fully appreciate the dangers that may follow upon the absorption of ammoniacal urine, especially after operations upon, or injuries to the urinary organs. In some subsequent investigations, MM. Gosselin and Robin\* found that benzoic acid best prevented the absorption of ammoniacal urine. As has been already stated, the benzoic acid is converted into hippuric acid, which unites with the ammonia and other bases forming hippurates.

The acid may be taken in doses of from one to six grams and is best given in syrup with some aromatic. The neutralization of the urine is generally accomplished within seven or eight days.†

*Excessive Acidity.*—It now remains for us to consider undue acidity of the urine. The acid may be so much in excess as to cause a burning pain during micturition and the patient says that his urine "scalds." In these cases the daily excretion is small. The undue acidity may be relieved with certainty by the administration of alkalis. In the selection of medicines for this purpose, those should be selected which least disturb the stomach and bowels. In this respect individual peculiarities are at times very marked. Thus, although the acetates and citrates are generally entirely unobjectionable, in some persons they invariably produce nausea, while the carbonates cause no derangement of the stomach. The administration of

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\* Archives Générales de Médecine, November, 1874.

† British and Foreign Medico-Chirurgical Review, April, 1875.

tartrates is generally followed by more or less purging. Water used in these cases in large quantities is an antacid, since it dilutes the urine and in this way prevents the irritation. Indeed, in many instances the insufficient amount of water drunk is the sole cause of irritation of the urinary passages.

When the highly acid urine contains free uric acid or calcium oxalate, or both, the administration of nitro-muriatic acid will, in many instances, prove very beneficial. This acid acts upon the stomach and liver, improving digestion and consequently rendering the changes in the food more complete, and converting the excess of uric acid and oxalic acid into urea and carbonic acid. The effects of the nitro-muriatic acid are due to its oxidizing and not to its acid properties.

#### SPECIFIC GRAVITY.

§ 143. *Methods of Ascertaining.*—Since the urine consists of water holding in solution certain solids, its weight can never be as light as that of an equal volume of pure water; also since the difference between the weights of equal volumes of urine and pure water will depend upon the proportion of solids contained in the former, the more concentrated a specimen of urine is, the higher will its specific gravity be. The most accurate method of determining the specific gravity of the urine consists in weighing a certain volume of the specimen at a certain temperature and dividing this weight by that of an equal volume of water at the same temperature. For this purpose, the urine and water may be measured and weighed in any small, clean flask or bottle; but it is more convenient to use a specific gravity bottle or pycnometer, which is a small bottle having a long stopper perforated with a capillary tube. This bottle is so made that when the stopper is accurately fitted it holds a certain number of c. c. (generally either 20 or 25) of water; since 1 c. c. of water weighs 1 gram, it contains the same number of grams. The pycnometer is filled to overflowing with the urine, the stopper is adjusted, the outside of the bottle wiped perfectly dry and the weight of the contained urine ascertained. Suppose that the pycnometer contains 25 grams of water and 25.5 grams of the urine under examination, then

if we consider the specific gravity of water as 1000, the specific gravity of the urine will be found from the following:

$$\frac{25.5 \times 1000}{25} = 1020.$$

*The Urinometer.*—The above method is, as has been stated, the most reliable and whenever scientific accuracy is desired, it should be used; but for the purpose of the physician, a more convenient method is desirable and it is furnished in the urinometer. This consists of a blown glass float with a bulb containing mercury for a weight and a shaft graduated so as to indicate the depth to which the instrument sinks in the fluid. The greater the proportion of solids contained in the urine, the less will the instrument sink and the more will its shaft project above the surface. The specific gravity of the urine is seldom above 1040, consequently the stem of the urinometer is graduated from 1000 to 1040. If the instrument be of convenient length and if only one be used, the lines on the stem indicating the depth to which the instrument sinks will be so close together as to render it difficult to decide within less than one degree as to which line coincides with the surface of the fluid; consequently the best form consists of two separate urinometers, the stem of one being graduated from 1000 to 1020, and that of the other, from 1020 to 1040. It is only necessary in the use of the urinometer to place the instrument in the urine and read off the specific gravity.

*Total Amount of Solids.*—After having found the specific gravity of a specimen, the weight of a given volume and the amount of solids contained in a given volume may be calculated. Suppose that during 24 hours, 1500 c. c. of urine are excreted and that the specific gravity of the specimen is 1020; now each c. c. of water weighs one gram, but this urine is 1.02 times as heavy as water and each c. c. of this urine weighs 1.02 grams and the weight of the 1500 c. c. will be found from the following:

$$1.02 \text{ grams} \times 1500 = 1530 \text{ grams.}$$

To ascertain exactly the total amount of solids contained in a specimen of urine is quite a difficult task and requires the use of complicated apparatus and much time. If a portion of

urine be evaporated, even at the temperature of the water-bath, much of the urea is decomposed and passes off as ammonia; consequently the weight of the residue would fall short of that of the total solids originally contained in the fluid. Again the residue which is obtained by evaporation of urine is very hygroscopic, rapidly absorbs water from the atmosphere and this introduces another source of error. It is thus seen that a simple, even though it may not be perfectly exact, method of ascertaining the total amount of solids in the urine is desired. It has been ascertained as the result of numerous experiments made with the greatest care that if the specific gravity of a specimen of urine be less than 1018, the total amount of solids in 1000 c. c. of that urine will be represented by the product obtained by doubling the last two figures of the specific gravity considered as a whole number.

Suppose amount of urine for 24 hours = 1500 c. c.

Suppose the specific gravity = 1015.

Then total solids in 1000 c. c. =  $15 \times 2 = 30$  grams.

The total residue in 1500 c. c. is found by the following proportion:

1000 c. c. : 1500 c. c. :: 30 grams : x, or 45 grams.

It has also been found that if the specific gravity be above 1018, the total amount of solids in 1000 c. c. of the urine will be found by multiplying the last two figures of the specific gravity by 2.33.

Suppose amount of urine for 24 hours = 1200 c. c.

Suppose the specific gravity = 1020.

Then total solids in 1000 c. c. =  $20 \times 2.33 = 46.60$  grams.

The total residue in 1200 c. c. is found from the following proportion:

1000 c. c. : 1200 c. c. :: 46.60 grams : x, or 55.92 grams.

*Average Specific Gravity.*—It now remains to consider the average specific gravity of normal urine and the variations that may occur in the same and those which result from disease. From what has already been given with regard to the amount of urine excreted within 24 hours, it will be seen that it is both difficult and unwise to set up any absolute standard for

the specific gravity of normal urine; for as a rule the amount and specific gravity vary inversely. The urine passed after drinking much water is known as *urina potus*, is pale and of low specific gravity sometimes as low as 1003. That excreted during sleep is called *urina sanguinis*, is of a brighter color, more acid and of a higher specific gravity, generally from 1012 to 1025; while the urine excreted after much solid food has been taken is known as *urina cibi*, is generally not so bright nor so acid as the *urina sanguinis*, but of a higher specific gravity, generally from 1015 to 1030. From this, the necessity of collecting all the urine excreted during the 24 hours is evident.

The specific gravity of the mixed 24 hours urine may vary in health from 1015 to 1030, and these limits may be passed temporarily without indicating any serious disorder; but it will be safe to say that if the specific gravity of the 24 hours urine continues for several days or weeks to be above 1030 or below 1015, some pathological condition of the body is indicated. A possible exception to this rule is furnished by the urine of pregnancy; for during the latter months of gestation the urine generally becomes more dense, and may constantly have a specific gravity above 1030. If the urine be highly colored and of a high specific gravity, there is generally an excess of urea and some febrile affection is indicated. Pale urine of high specific gravity occurs in diabetes mellitus, and in this disease the density may be as great as 1060, such an increase being an indication of the progress of the disease.

Albuminous urine is generally of low specific gravity, and in parenchymatous inflammation the less dense the urine the more serious the indication, since it is evidence of the retention of a large amount of urea. In amyloid degeneration of the kidney the urine often becomes more dense as the disease progresses and in the last stages the specific gravity may be 1040 or higher. This is due to the diminished amount of urinary water, the excretion for the 24 hours sometimes not measuring 100 c. c. In renal cirrhosis, the specific gravity is less than 1020. In diabetes, insipidus and in hysteria, the urine is of low specific gravity, the urinometer in some cases registering only 1002.

## OTHER PHYSICAL PROPERTIES.

§ 144. *The Odor.*—The urines of different animals have characteristic odors which are due to volatile oils and in general resemble the odor of the fat of the animal. When recently passed, the odor is most perceptible, because the temperature of the specimen is higher than it subsequently becomes unless heat be applied, and because there is more of the volatile oil than there is after the urine has been passed for some time. The odor is often a valuable aid to one in determining whether a specimen be urine or not, and if so, the urine of what animal. In making this determination the fluid should be heated, and if necessary evaporated to dryness and the residue burned when, if the fluid be, or contains urine, the odor will be recognized.

Heating urine with nitric acid increases but also modifies the odor. Many articles of food and medicinal substances impart characteristic odors to the renal excretion. The internal use or even the inhalation of oil of turpentine produces in the urine the odor of violets. Asparagus imparts to the urine a peculiar and very disagreeable odor. Again the urine may have an abnormal odor as the result of pathological conditions, as the ammoniacal odor of cystitis and the peculiar fishy smell of some forms of albuminuria.

*The Taste.*—The urine of man has a bitter and a salty taste, the former being due to an organic principle, urochrome, and the latter to sodium chloride. In diabetes the taste is sweet, and it was by the application of this test that sugar was first discovered in the urine; while in icterus the taste is bitter from the presence of bile. Drinking the urine, which has been resorted to in cases of necessity, has been found to increase the thirst.

*The Temperature.*—Patients sometimes complain and say that their urine scalds; now the urine receives its heat from the body, and consequently, when in the bladder or when passing along the urethra, cannot be of a higher temperature than the surrounding tissues. The irritation caused is not due to the high temperature of the fluid, but may be caused by an inflamed or raw condition of the tissues, or by the excessive acidity of the urine,



or by both; for the latter not unfrequently produces the former. If the urine be excessively acid, the administration of an alkali and an increased consumption of water will soon relieve the difficulty; while if the tissues be inflamed, injections suitable to the case may be used.

*Deposits.*—The only deposit occurring in normal urine within 24 hours after its emission, (except when the urine decomposes sooner, as it does in very warm weather) is a faint cloud consisting of epithelial debris from the mucous membrane of the urinary passages. This may be recognized by the ease with which it is distributed on agitating the fluid and by its insolubility in acids. After a greater or less length of time after emission, any urine will become alkaline and deposit phosphates, or before this period has been reached, it may undergo the acid fermentation and deposit calcium oxalate and uric acid. But any deposit, other than mucus, occurring in urine within 24 hours after emission must be regarded as pathological. Moreover it is not necessary that such a deposit should be visible to the unaided eye; thus calcium oxalate may be deposited in large quantity and the urine appear perfectly normal. The discussion of the various deposits will be given under the several substances forming such deposits.

#### UREA,— $\text{CH}_4\text{N}_2\text{O}$ .

§ 145. Urea is the principal organic constituent of normal urine and exists in the blood and in various transudates. On account of its free solubility urea never forms a spontaneous deposit in the urine. It has been found in the amniotic fluid, the aqueous humor, lymph and chyle. Urea may be prepared synthetically or obtained from the urine.

*Preparation.*—(1) To some urine (from 200 c. c. to 500 c. c.) add the baryta mixture (made by mixing two parts of a saturated solution of barium hydrate with one part of a saturated solution of barium nitrate) as long as the precipitate increases. Remove this precipitate, which consists of barium phosphate and sulphate by filtration; concentrate the clear filtrate to a syrup on the water-bath; extract this syrup with alcohol; filter and evaporate the alcoholic extract to dryness on the water-

bath; extract this residue with absolute alcohol; again filter and evaporate at  $100^{\circ}$ ; on cooling, this residue will be found to consist of a mass of crystals of urea. By this method quite pure urea is obtained; but the process is attended with considerable loss, the urea being decomposed during evaporation.

(2) Urea may also be prepared from human urine by the following process: Concentrate from 100 c. c. to 500 c. c. of urine of high specific gravity and of an acid reaction to a small volume on the water-bath; to this syrup kept at  $0^{\circ}$ , add an equal volume of strong nitric acid. After a short time a mass of crystals of nitrate of urea forms. Collect these upon a filter and dry by pressing them between folds of blotting paper; then dissolve them in water and add barium carbonate with stirring as long as gas is liberated. Barium nitrate is formed and the urea is set free. Evaporate the mixture to dryness at  $100^{\circ}$  and extract the residue with absolute alcohol; filter and concentrate the filtrate and allow to stand in a cold place when urea crystallizes.

(3) Feed a dog for several days upon all the lean meat that it will eat. Collect the urine excreted by the animal during this time; concentrate it to a syrup on the water-bath; extract with alcohol; filter and again concentrate; extract this residue with absolute alcohol; filter, concentrate and allow to stand in a cold place when urea crystallizes.

(4) Urea is ammonium cyanate and may be prepared artificially in various ways. Mix two parts of dry potassium ferrocyanide with one part of the black oxide of manganese on a thin iron plate; apply heat until the mixture burns thoroughly; extract the cooled residue with water; to the filtered aqueous extract add one and one-half parts of ammonium sulphate; evaporate the mixture to dryness and extract the residue with absolute alcohol which dissolves the ammonium cyanate, or urea. On concentrating the alcoholic extract, urea forms in crystals.

(5) Fuse potassium cyanide mixed with lead oxide; extract the residue with water, add ammonium sulphate and proceed as above.

*Properties.*—Urea forms in fine, long, four-sided prisms which

are terminated at each extremity by short pyramids. The crystals are not hygroscopic, are freely soluble in water and alcohol, insoluble in anhydrous ether. The dry crystals may be heated to  $110^{\circ}$  without decomposition, but in solution, especially in the urine and more rapidly if the urine be alkaline, urea is decomposed with the formation of ammonium carbonate.

*Combinations.*—Urea is a base uniting with acids to form characteristic salts. If urine or water containing as much as 10 per cent. of urea be treated with an equal volume of strong nitric acid and the mixture be kept in a cold place, nitrate of urea will be precipitated. This compound results from the simple combination of the acid with the base and is represented by the formula,  $\text{CH}_4\text{N}_2\text{OHNO}_3$ . It crystallizes in rhombic plates or prisms and is sparingly soluble in cold, more freely in hot water, insoluble in strong nitric acid. The study of this salt is important, since the detection of urea in many fluids depends upon the formation and recognition of these crystals.

If a solution containing as much as 20 per cent. of urea be treated with oxalic acid, the oxalate of urea is formed and also crystallizes in rhombic tables. These crystals contain water and are represented by the formula,  $\text{CH}_4\text{N}_2\text{OH}_2\text{C}_2\text{O}_4 + 2\text{H}_2\text{O}$ . This salt is sparingly soluble in cold water and consequently is sometimes recognized by its crystalline form in the microscopical examination of concentrated urine.

Urea combines not only with acids but also with some bases; thus, if a solution of urea be treated with one of mercuric nitrate a white precipitate consisting of the oxide of mercury, urea and nitric acid is thrown down. The exact proportion of these constituents contained in the precipitate depends upon the concentration of the solutions and the amount of free acid present. According to Liebig, there are three different compounds formed with mercuric nitrate and urea; the first contains four equivalents of mercuric oxide, the second three, and the third one, to one equivalent of urea. If any of these compounds be suspended in water, the mixture treated with  $\text{H}_2\text{S}$  gas and the mercury sulphide removed by filtration, nitrate of urea will exist in the filtrate and may be recognized on concen-

tration by its microscopical appearance. Upon the fact that urea is precipitated by mercuric nitrate depends the ordinary method of estimating the quantity of the former.

*Decomposition.*—Urea is decomposed in the presence of putrid animal matter, the decomposition consisting in the assumption of a molecule of water and the formation of ammonium carbonate. This change has already been referred to and represented by an equation as the cause of the ammoniacal odor of putrid urine. If a solution of urea be treated with one of sodium hypobromite, the former is rapidly decomposed with the formation of water, carbonic acid gas and nitrogen.  $\text{CH}_4\text{N}_2\text{O} + 3\text{NaBrO} = 3\text{NaBr} + \text{CO}_2 + 2\text{H}_2\text{O} + 2\text{N}$ . If there be an excess of the alkali present, the carbonic acid unites with the base forming a carbonate, while the nitrogen passes off as a gas and may be collected, measured and the amount of urea calculated. Upon this fact depends the hypobromite method of estimating urea. Similar decompositions are caused by the action of nitrous acid and hypochlorite of soda on urea.

§ 146. *Physiology.*—Urea is the principal product of the retrograde metamorphosis of nitrogenous material. It was for a long time supposed to be a product of "vital force," but in 1828 Wöhler succeeded in preparing urea artificially, and to-day the chemist manufactures as good an article in his retort as is formed in his body. It is the great vehicle for carrying off the waste nitrogenous matter of the food and is probably formed in various parts of the body. Indeed some urea is found in the small intestines and its presence there is a result of the processes of digestion; so that even before the food has been absorbed, a part has been so changed as to be of no farther value either in the liberation of force by farther changes in itself or in building up tissue. It is quite evident that in the liver more urea is formed; the nitrogenous parts of the food being broken up into urea, uric acid and other less highly oxidized nitrogenous compounds on the one hand and on the other, into non-nitrogenous substances, such as glycogen.

A true knowledge of the physiology of urea can be obtained only by a study of the value of nitrogenous foods; because,

with the exception of insignificant traces, the excreted nitrogen of the system is found in the urine as urea and uric acid, principally as the former. The old theory, advanced by Liebig, and which stood unquestioned for a long time, divided foods into two principal classes, viz., the heat producing and the tissue forming. According to this theory, the principal or sole use of nitrogenous food was to build up muscular tissue; consequently, it was held that muscular exertion was rendered possible by the liberation of force resulting from the disintegration of muscular tissue itself and that the amount of urea excreted within a given time depends upon the amount of muscular exertion put forth and is independent of the kind and amount of food. In order that the theory of Liebig may be fairly represented and understood, the following quotation will be given: "Boiled and roasted flesh is converted at once into blood; while the uric acid and urea are derived from the metamorphosed tissues. The quantity of these products increases with the rapidity of the transformation in a given time, but bears no proportion to the amount of food taken in the same period. In a starving man, who is in any way compelled to undergo severe and continued exertion, more urea is secreted than in the most highly-fed individual if in a state of rest."

After some years, thoughtful men began to question these assertions, which seem to have been made without any substantial basis of experimentation. Lehmann soon found that the quantity of urea and uric acid excreted does depend to some extent upon the food. Fick and Wislicenus proved by direct experimentation that work could be done upon a non-nitrogenous diet and without increased disintegration of the substance of the muscle. Drs. Parkes, Pavy and Haughton have experimented upon this subject and reached the same general conclusions. In order to show the effect of the kind and amount of food upon the excretion of nitrogen, I will give some experiments made upon myself and relating to this subject. For fifteen days I accurately measured and weighed all my food, taking the same both in quality and quantity each day. During the last five of these days I collected the urine

and estimated the amounts of urea and uric acid and calculated from these the quantity of nitrogen excreted. The relation between the nitrogen of the food and that of the urea and uric acid is shown by the following:

Nitrogen of the daily food=225.107 grains.

Nitrogen daily excreted =230.205 grains.

It will be seen from this that after the continued consumption of the same amount of nitrogenous food for 10 days, the nitrogen of the food and that of the excretions are practically the same; the difference of about five grains being slight. At the expiration of the first period of 15 days, the daily amount of nitrogenous food was diminished and this smaller quantity was taken for a second period of 15 days. During the last five of these days, the urine was again collected and the amounts of urea and uric acid estimated; the relation between the nitrogen of the food and that of the urea and uric acid is represented by the following:

Nitrogen of the daily food=155.899 grains.

Nitrogen daily excreted =155.394 grains.

It should be stated that during both of these periods, my food consisted of inorganic, starchy, fatty and nitrogenous articles, and that the change from the first to the second period simply consisted in omitting a part of the nitrogenous food. During both of these periods but little muscular exercise was taken. The waking hours of the day were spent in reading and conversation. I walked to the university library and back to my room each forenoon and to the laboratory and back each afternoon, in all not one mile per day. The little physical exercise with the reading kept me from feeling dull. I retired each night promptly at ten o'clock and arose at seven o'clock.

These experiments are certainly sufficient to convince one that the amount of urea excreted within a given time does depend largely upon the food. Indeed this is the universal conclusion of those who have experimented upon this subject.

Now during a third period which embraced only 5 days, I took the same amounts of inorganic, starchy and nitrogenous

foods as in the second period, but increased my fatty food by one ounce of butter. During each of these five days, in addition to the little physical exercise taken during the other periods, I walked 11 miles on a rough railroad bed at the rate of 3 miles per hour, passing the remainder of the time as during the two preceding periods. The object of this period of experimentation was to ascertain whether the additional exercise would increase the excretion of urea. The urea and uric acid for each day were estimated as before. The relation between the nitrogen of the food and that of the urea and uric acid is represented by the following:

Nitrogen of the daily food=155.899 grains.

Nitrogen daily excreted =149.129 grains.

From this, it is seen that the amount of nitrogen excreted as urea and uric acid was not increased by muscular exertion\*.

The experiments of Prof. Haughton and others prove the same. Prof. Haughton found that under ordinary conditions, and when his physical exercise never equaled a walk of five miles per day, his average excretion of urea was 501.28 grains; while the average amount of urea excreted during five days, in which the average amount of physical exercise was equal to a walk of 20.74 miles, was 501.16 grains†.

From the many experiments made upon this subject and the great similarity of the results obtained, we are justified in asserting that in a healthy man, the amount of urea is not increased by any reasonable amount of muscular exertion. Of course, everyone knows that in certain diseased conditions, the muscular tissue of the body is disintegrated and increases the amount of urea; but we are now studying physiological and not pathological conditions. For this reason, as Dr. Pavy has shown, the experiments of Prof. Flint on Weston are of but little value; for, to quote the words of Prof. Flint, "At 10:30 P. M. of this day (the fourth of experimentation) Weston broke down completely. He could not see the track, and was

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\* For further details in these experiments, see *The Physician and Surgeon*, January, 1879.

† *Medicine in Modern Times*, p. 125 et seq.

taken staggering to his room, having reached, apparently, the limit of his endurance." We should not feel warranted in deducing any physiological laws from experiments upon a man who "could not see, and who was taken staggering to his room."

Dr. Edward Smith has shown\* by a long series of experiments, which are an honor to the profession, that muscular exertion does increase the amount of carbonic acid exhaled, and physiologists are now generally united on this point. We are to measure the amount of physical exercise by the amount of carbonic acid exhaled, and not by the amount of the urea and uric acid excreted; moreover, there is not proof sufficient to justify the old idea that every part of the human body is replaced every few years by new material.

*Effects of Medicines upon the Excretion of Urea.*—Hundreds and probably thousands of experiments have been made in order to determine the effects of medicinal agents upon the elimination of urea. Let us see how some or the majority of these experiments have been made and then we can judge of their value. In one medical journal, I find that Dr. ——— estimated the amounts of urea passed by three healthy persons and three others who had renal disease in 24 hours without medication, and then estimated the amounts of urea passed by the same persons in 24 hours beginning with or succeeding a jaborandi sweat, and found that in five of these persons the amounts of urea were increased, while in the other, the amount was slightly decreased. The conclusion drawn is that jaborandi increases the amount of urea excreted within a given time. This drug may have the above effect, but the conclusion is certainly not warranted by the experiments. It is not stated whether these persons ate much, little or nothing during these days. All experiments upon the elimination of urea, which do not consider the amount of nitrogenous food, are of but little value. Another way of making these experiments is to estimate the amount of urea passed during a number of consecutive days without medication, then for the same number of days

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\*Cyclical Changes in the Human System.



with medication, then again for the same number of days after medication; take the average for each period and compare results. Even this method is far superior to the first one given; for in the first, only one day without medication, and with or after medication was taken into consideration and consequently errors, which may arise in this method, stand a chance of being corrected by the extension of the time. But even in the second method, the experimenter generally *presumes* that he eats *about* the same amount of food each day. Now presumptions do very well when no more accurate knowledge is possible, but it is hardly wise for the *scientific* physician of this day to build upon the sands of presumption when the rocks of certainty not only lie near by, but with regard to foods (thanks to Payen, Letheby and others who have given us the per cent. of nitrogen and other constituents of the most common foods) they are already carved and fitted.

*Circumstances affecting the Excretion of Urea.*—It will be well to consider some of the circumstances which affect the excretion of urea. The effects of variations in the kind and amount of food have already been sufficiently referred to. Any agent which interferes with or improves digestion may correspondingly diminish or increase the amount of urea excreted in a given time. In cases of indigestion an abnormally large proportion of material may pass from the body as *faeces*; consequently, so much nitrogen escapes conversion into urea and the amount of the latter is necessarily lessened. Again if the food is only partially or poorly digested and is absorbed in this condition, the process of oxidation is retarded to a corresponding extent and that which should form urea will pass from the body as uric acid or as the yet less highly oxidized substances, xanthin and hypoxanthin. For a similar reason, the excretion of urea is influenced by any thing which helps or hinders the processes of oxidation which normally occur in the body; thus in diseased conditions of the lungs, the amount of urea will often be found sensibly diminished. This constituent of the urine may also be increased by an increased consumption of water; in this case the blood is more nearly deprived of its waste

material as it passes through the kidneys, the water extracting the soluble substances. The heat of fever consumes the muscular tissue of the body and consequently augments the quantity of urea. Again the urea may be retained in the body after it has been formed; this is the case in the last stages of structural diseases of the kidneys. In diseased states, it may also pass from the body through other avenues than the kidneys; thus cholera stools and the perspiration of those who suffer from renal disease may contain urea.

The formation of urea in the body is not only a normal but a necessary process and if the material for its formation is not furnished as food, then the muscular tissue of the system will be disintegrated and supply the place of the food. The products of the retrograde metamorphosis of nitrogenous material are necessary to the maintainance of the functions of life; but these, like other stimulants, must not be allowed to accumulate in excessive quantity in the blood; for in large quantities they are true poisons, producing serious and often fatal disturbances of the nervous system as seen in the convulsions of uræmia. If an animal be starved, the urea for a while rapidly diminishes and then remains stationary until death. The production of this substance falls to its minimum and as soon as this fails, death results.

The relation between urea and uric acid has been worked out by experiments, both in the test tube and in the body. If uric acid be given an animal, it increases the amount of urea; thus, Neubauer found, that by giving rabbits from 2 to 3 grams of uric acid, the amount of urea was increased from 2.1 to 4.2 grams. This same experiment has been verified by myself upon many animals.

The amount of urea excreted by a healthy man living upon ordinary mixed diet will vary from 18 to 36 grams for the 24 hours. An amount, which at one time may be abnormal, may at another time be perfectly normal.

§ 147. *Pathology.*—From what has been said concerning its physiology, it will not be difficult to understand why urea is in excess in some diseases and deficient in others. By the expres-

sion, "an excess of urea," one of two things might be understood. These are, (1) an excess in proportion to the water and (2) an absolute excess for the 24 hours. A specimen of urine may contain an excess of urea as understood in the first sense and a deficiency in the second. We decide as to whether urea is in excess in proportion to the water as follows: Place a few drops of the urine under examination on a glass slide, add an equal volume of strong nitric acid and set aside in a cool place for five minutes, at the expiration of this time, if urea were in excess in proportion to the water, a mass of crystals of nitrate of urea will have formed and be plainly visible to the unaided eye; while if there be but a normal or deficient proportion of urea, no crystals will be seen. In order to test for a deficiency, evaporate some of the urine to one-half its volume, place a few drops of this on a glass slide, add nitric acid and allow to stand as before. If at the expiration of this time no crystals are seen, the urea is deficient in proportion to the water. In all cases in which there is an excessive proportion of urea, the specific gravity is necessarily high. Of course if the proportion of urea be excessive and the amount of urinary water also abnormally large, the absolute quantity of urea for the 24 hours will be excessive. In those cases where there is only a relative deficiency of urinary water, the treatment has already been discussed in considering the amount of urine, and further remarks made here will apply to those cases in which there is an excessive excretion of urea within a given time. The exact amount can be ascertained only by quantitative estimation.

As a rule, the higher the temperature of the body, the greater the quantity of urea that is formed. This is true in febrile diseases; for instance in typhoid fever the daily excretion of urea may reach 75 grams or even higher and this too, when but little food is taken, and consequently the enormous excretion of urea represents disintegration of muscular tissue. In typhus fever, the temperature of the interior organs of the body rises to  $105^{\circ}$  and higher, and this excessive heat is the result of the combustion of the body itself. If we could know the cause of the increased temperature or if we could under-

stand why it is that the tissue is so rapidly disintegrated then the removal of the cause would be the rational treatment; but we do not know these things, consequently the physician either does nothing at all or supplies other fuel for the fires of the fever. As yet we know no way of quenching these fires, consequently we furnish fuel as wine and beef-tea in order to preserve the body itself from combustion.

The amount of urea is increased in intermittent fever and in inflammatory diseases as in pneumonia, also in the exacerbations of some chronic diseases and in the paroxysms of epilepsy. The increase of urea in diabetes mellitus affords an interesting study. In health much of the force necessary to the maintenance of animal heat and the exercise of the body is furnished by the combustion of starchy food which is transformed or burned into carbonic acid and water. In diabetes mellitus the starchy food escapes combustion and the nitrogenous material must be consumed in its stead. For this reason, it is that the diabetic patient has a voracious appetite and for the same reason he gradually fails. Says Prof. Haughton, "The diabetic patient resembles a racing steamboat on the Mississippi, whose supply of coals is exhausted and whose cargo furnishes nothing better than lean pork hams, to throw into the furnace to maintain the race. It cannot be wondered at, that our poor patient, under such disadvantageous conditions, fails to keep in the front."

In Asiatic cholera, the conditions are the reverse of those of fever. In this disease the temperature is diminished, the heat is not sufficient to maintain life, and the circulation is impeded; consequently there is a deficient formation of urea. It is useless to supply material for combustion because it cannot circulate through the system. It is desirable to restore the due amount of heat to the body and thus render oxidation possible.

Deficiency of urea may be due either to decreased formation or to diminished elimination; while the former is undoubtedly the cause of the small amount of urea found in the urine of cholera, the latter condition prevails in structural diseases of the kidney. Retained urea acts as a poison and if the retention

be marked and be not speedily relieved, death soon follows. The symptoms produced by retained urea are known as those of *uræmia*. The nervous system is affected and convulsions often repeated may follow. It is questioned by some whether these symptoms are produced wholly by retained urea or in part by other causes. In the breath of some persons suffering with these convulsions, ammonium carbonate has been obtained and this has led some to believe that the nervous disturbance was caused by ammonium carbonate and the term, *ammonæmia* has been suggested to designate this condition. The truth no doubt is that in some instances the symptoms are due to retained urea; in others, to the reabsorption of ammonium carbonate from the urinary passages; and in still others, to the retention or undue formation of other nitrogenous substances; or the symptoms in a particular case might be due to all these causes combined. Of course, the ammonium carbonate arises from the decomposition of urea. We must recognize the fact that the nitrogenous constituents of our food, during their passage through our body, undergo certain changes whereby a series of new substances is produced; each of these is one degree nearer the confines of inorganic nature than its predecessor and all of these in moderate quantities are valuable as nerve stimulants, but are dangerous when allowed to accumulate in the system. Our ancestors ate much more nitrogenous food than we do, and consequently the physician of the past resorted to bleeding more frequently than the physician of the present would be justified in doing. Bleeding was then found beneficial because by this means the excess of nitrogenous material was quickly and surely removed.

The so-called uræmic symptoms of pregnant women are variously explained by different writers. Be the cause what it may, the urine usually contains more or less albumen and a deficiency of urea for some time before the attack. In the most of these cases, no examination of the urine is made and the true condition of the patient is not suspected until her frame is convulsed by the poison. All of this might have been foreseen and prevented in the great majority of cases by a timely

examination of the urine. The urine of every pregnant woman should be repeatedly examined and the physician of the present day, who has the care of such a patient and fails to make such examinations, is guilty of criminal negligence.

URIC ACID,  $\text{—C}_5\text{H}_4\text{N}_4\text{O}_3$ .

§ 148. *Occurrence.*—Uric acid is the principal constituent of the urine of reptiles and birds. In the urine of carnivora and of man, it is found in small quantities; while in the urine of herbivora, it is replaced by hippuric acid. Uric acid in small quantities is a normal constituent of the liver, spleen, lungs, muscle, brain and blood. In the lower animals, an accumulation of uric acid in the blood can be produced by ligating the ureters or by extirpation of the kidneys. Uric acid is not unfrequently found in various transudates; while it is found in man in largest quantities in gravel and calculi of either the free acid or urates.

*Preparation.*—(1) Uric acid is obtained with ease and in a state of purity from the urine of serpents as follows: Dissolve the powdered excrement in a hot dilute solution of sodium hydrate (one part of caustic soda dissolved in ten parts of water); boil this solution as long as the odor of ammonia is given off; filter while hot; treat the filtrate with a current of carbonic acid gas until a granular precipitate falls; allow to stand for 24 hours; collect the precipitated acid sodium urate upon a filter; wash it with a little cold water, then redissolve in a hot dilute solution of sodium hydrate and add hot dilute hydrochloric acid sufficient to make the mixture strongly acid. Allow this to stand for 24 hours, when pure, white uric acid will be deposited in rhombic tablets. The supernatant fluid may be decanted, the crystals washed with water then with alcohol and dried. If guano be treated as above, uric acid will be obtained in larger quantity than from the urine of serpents; but it will not be so pure.

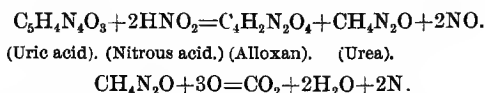
(2) Uric acid is obtained from the urine of man by the following method: To some (not less than 200 c. c.) clear urine of specific gravity not less than 1025, add 5 per cent. of hydrochloric acid and allow to stand for 24 hours in a warm place.

The hydrochloric acid combines with the bases and sets uric acid free. At the expiration of the 24 hours, the specimen will be found to contain a brick-dust deposit and reddish crystals will be observed floating upon the surface of the fluid and adhering to the sides of the beaker. By means of a glass rod with a small piece of rubber tubing drawn over one end, gently agitate the fluid and rub the crystals from the sides of the vessel. All the crystals will then subside. Decant the supernatant fluid and add to the sediment a little water; agitate gently and again decant; then wash by decantation with alcohol; collect the crystals on either a filter or watch-crystal and dry at 110°. As thrown down from the urine, the crystals of uric acid are more or less colored. If it be desired to obtain uric acid from urine of low specific gravity, such urine should be concentrated and then treated as above. If albumen be present it should be removed by precipitation with a little acetic acid, heat and filtration, and the filtrate concentrated and treated as above.

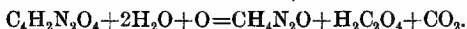
*Properties.*—Pure, dry uric acid is a white, odorless and tasteless powder. The pure acid does not crystallize so readily nor take such a variety of shapes as that which contains more or less coloring matter. It has already been stated that when normal urine undergoes the acid fermentation, uric acid is liberated from its combination with the bases; in these cases, the acid is deposited in beautiful crystals of a reddish or brown color. The most perfect crystals of uric acid are long rhombic plates; but in the majority of instances, these are greatly modified. Frequently in the urine, the crystals will be found arranged in bundles or rosettes which, not unfrequently, are visible to the unaided eye. These crystals contain no water and are insoluble in alcohol and cold dilute acids and practically insoluble in water, 1 part of the acid requiring 1800 parts of boiling, and 14000 parts of cold water for solution.

*Alloxan.*—To a few drops of nitrous acid in a small dish or watch-crystal, add some pure uric acid. A brisk effervescence immediately occurs and after standing for a few moments, a white substance will remain suspended in the acid. Microscop-

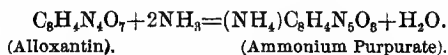
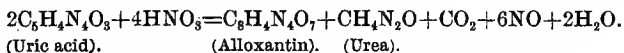
ical examination will show that this substance, which is *alloxan*,  $C_4H_2N_2O_4$ , is composed of beautiful colorless cubes or octohedrons. The nitrous acid has oxidized the uric acid forming alloxan, urea and nitrous oxide; while the urea is immediately decomposed into carbonic acid, nitrogen and water. These changes are represented by the following equations:



The alloxan, after standing in the acid for some time, is gradually oxidized into oxalic acid, carbonic acid and urea, and since nitrous acid consists of nitric acid containing the oxides of nitrogen, as soon as these oxides are decomposed, the urea combines with the nitric acid forming the nitrate of urea. The crystals of nitrate of urea will be recognized on microscopical examination. The change by which alloxan is converted into urea, oxalic acid and carbonic acid is represented in the following equation:



*Alloxantin*.—To some uric acid in a small evaporating dish add nitric acid; place the dish on the water-bath and heat to dryness. If this experiment be made in a laboratory where there is much ammonia in the atmosphere, the residue left on the sides of the dish as the nitric acid evaporates will take a beautiful crimson tint; otherwise a white residue remains and becomes crimson on the addition of a little ammonia or on being placed under a bell jar filled with the vapor of ammonia. The uric acid has been changed by the action of the nitric acid into alloxantin,  $C_8H_4N_4O_7$ , and the alloxantin unites with ammonia forming the purpurate of ammonia. These changes are represented thus:



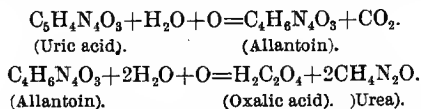
*Murexid Test*.—Ammonium purpurate is also known as murexid, and consequently the above reaction is known as the



murexid test. It forms a delicate means of detecting the presence of uric acid and is consequently of great value in the analysis of urine. Certain precautions are necessary in its application. In the first place, other organic matters must be removed as far as possible; because some will give a similar color when carried through the process and others will interfere with the development of the color when uric acid is present. In applying this test to urinary deposits the supernatant fluid should *never be evaporated*, but should be removed either by decantation or filtration. The sediment should then be washed by decantation or on the filter with a little cold water and then with alcohol. Neither of these will dissolve either free uric acid or acid urates, and in these conditions only is uric acid deposited in the urine. The washed sediment is then placed in a clean porcelain dish, nitric acid added and the mixture heated to dryness on the water-bath. To the residue, kept warm on the water-bath, add a little ammonium hydrate, when if uric acid were originally present, either as free acid or as a urate, the crimson color will be developed. The addition of an excess of ammonium hydrate must be avoided and if the quantity of material under examination be small, it is better to simply blow a spray of ammonia over the dish.

*Allantoin*.—To a solution of uric acid in potassium hydrate, add a solution of potassium permanganate until the mixture becomes slightly colored; filter and allow the filtrate to stand in a cool place (heat must not be applied at all in this process) for twenty-four hours, than add acetic acid sufficient to produce a decidedly acid reaction and allow to stand for twenty-four hours longer, when crystals of *allantoin*,  $C_4H_6N_4O_3$ , will be deposited. These may also be obtained by adding lead peroxide to a mixture of uric acid and water, keeping near the boiling point for some time, filtering and allowing the filtrate to cool. Allantoin forms in small glistening, transparent prisms, which are insoluble in cold alcohol and ether, soluble in hot alcohol, sparingly soluble in cold water, more freely in hot water. This substance takes its name from the fact that it was first discovered in the fluid surrounding the foetus, and was supposed to

be secreted by the allantoinic membrane. Later it has been found in the urine of calves and in that of new-born children. According to Hermann and others, allantoin in small quantity is a normal constituent of the urine of the adult. It is probably a link between uric acid and urea, and in this connection the following equations, representing the conversion of uric acid into allantoin, and of the latter into urea and oxalic acid will be of interest:



*Combinations of Uric Acid.*—Uric acid is soluble in the caustic alkalis (freely in sodium and potassium hydrates, sparingly in ammonium) forming urates. It is in this state that uric acid exists normally in the blood and urine; for in these free, uric acid is found only as the result of pathological conditions. Uric acid is also soluble in solutions of the phosphates, carbonates, borates, lactates, and in citrates of sodium and potassium (not of ammonium). If a solution of uric acid in caustic potash or soda be treated with a current of carbonic acid gas, a precipitate consisting of an acid urate is thrown down. The deposits of urates, which occur so frequently in the urine, are acid urates; consequently the study of these salts is of value.

*Sodium Urate.*—The most common deposit in the urine is the acid urate of sodium,  $\text{NaC}_5\text{H}_3\text{N}_4\text{O}_3$ . It together with free uric acid forms the so-called lateritious sediments. Under the microscope, only amorphous flakes are generally seen: while the deposit readily clears up on the application of heat. Its ready solubility on being warmed affords an easy means of recognition, for the acid urate of potassium does not dissolve until the temperature is raised nearly to the boiling point; while a deposit consisting of the acid urate of ammonium does not dissolve until the mixture is brought to the boiling point. The acid urate of sodium may be produced artificially by treating a solution of uric acid in sodium hydrate with a current of carbonic acid gas, until a precipitate forms. Prepared in this way, it appears under the microscope in granules. It may also be

obtained by mixing a hot solution of uric acid in sodium hydrate with a solution of the bicarbonate of sodium, or by boiling uric acid in a solution of sodium phosphate. By either of these methods it forms in fine needles which often are arranged in balls and bundles. The salt is soluble in 1200 parts of cold, and 125 parts of hot water.

*Potassium Urate.*—The acid urate of potassium,  $\text{KC}_5\text{H}_3\text{N}_4\text{O}_3$ , resembles the corresponding salt of sodium, and may be prepared in a like manner. It occurs as a urinary deposit, and requires 800 parts of cold, and 80 parts of boiling water for solution. Besides these two salts, quadriurates of potassium and sodium occur in the urine and may be prepared by adding acetic acid to solutions of uric acid in sodium and potassium hydrates until a feebly acid reaction is obtained. Both sodium and potassium quadriurates are amorphous. The potassium salt has the formula,  $\text{KC}_6\text{H}_3\text{N}_4\text{O}_3 + \text{C}_6\text{H}_4\text{N}_4\text{O}_3$ .

*Ammonium Urate.*—The acid urate of ammonium,  $\text{NH}_4\text{C}_5\text{H}_3\text{N}_4\text{O}_3$ , occurs as an urinary deposit and often in dumb-bells or in balls of radiating needles, especially in ammoniacal urine. It may be obtained by adding ammonium hydrate to uric acid suspended in boiling water, when it appears in fine needles.

*Calcium Urate.*—Acid urate of calcium is rarely met with in urinary deposits, when it appears in fine needles which may be mistaken for tyrosin. It may be obtained by adding calcium chloride to a solution of uric acid in sodium or potassium hydrate, when the acid urate of calcium is precipitated in an amorphous form.

§ 149. *Physiology.*—Like urea, the uric acid, which is excreted in health, results from the food; while in disease, it may be due to disintegration of the muscular tissue of the body. It is therefore, evident that an increased consumption of nitrogenous food will increase the amount of uric acid formed; consequently in many instances, it is found that the urea and uric acid vary in the same ratio. But there are other circumstances which may cause the amounts of these substances to vary inversely and to these will we now direct our attention. It has already been seen that uric acid outside

the body is converted by means of oxidizing agents into urea. The former is the result of less perfect oxidation. That deficient oxidation increases the amount of uric acid and correspondingly decreases that of urea is proved by every fact which we know concerning the variations of the amounts of these substances. Wine drinking increases the quantity of uric acid, because the alcohol is more readily acted upon by the oxygen of the oxyhæmoglobin and the nitrogenous constituents of the food escape combustion. Again, constant drinking of wine leads to disease of the liver and this organ plays an important part in splitting the albumen of our food into carbohydrates, urea and uric acid. Again in diseases of the lungs, when but an insufficient supply of oxygen reaches the blood, the quantity of uric acid is increased and that of urea correspondingly decreased. In venous stasis, the same result follows because the blood is not oxidized sufficiently. In indigestion the processes of retrograde metamorphosis are retarded and the nitrogen leaves the body just so much farther removed from urea. Those living in poorly ventilated houses excrete an excess of uric acid and calcium oxalate; the same is true of those who take but little physical exercise.

It is said that uric acid cannot result from imperfect oxidation, because birds take in large quantities of oxygen in proportion to their body weight and yet their urine consists principally of uric acid. We should remember that the products of chemical changes depend upon the conditions under which they take place. Oxygen and uric acid are not the only essentials for the production of urea; the conditions must be favorable. A diamond may be exposed to the oxygen of the atmosphere for centuries and yet remain unchanged, yet is this proof that the product of the oxidation of the diamond is not carbonic acid? Large quantities of arsenic may be given to a pig without causing death; would it be wise for a physician to decide from this, that the same amount of arsenic would not injure his patient? The surrounding conditions of the nitrogenous food in the body of birds are very different from those attending the changes of the same food in man and as long as

these differences exist, it is very unwise to expect that the products of the changes in the two will be identical.

The average daily excretion of uric acid by a healthy man is about .4 of a gram. Free uric acid is not a constituent of normal urine; consequently when we speak of the amount of uric acid in normal urine, we refer to that existing in a combined state. It is combined with sodium, potassium, ammonium and calcium. Since free uric acid does not occur in normal urine and the urates, when normal, are in solution, any deposit of either shows that there is some abnormality.

§ 150. *Pathology.*—Free uric acid may be deposited from any of the following causes: (1) The urine may be unduly acid, the stronger acids taking up the bases and setting the uric acid free; (2) there may be an absolute excess of uric acid formed, so that the normal amount of bases is not sufficient to take up all the acid; (3) the proportion of alkaline bases may be abnormally decreased.

Not unfrequently, either, while the urine is in the passages or after it has been passed, by a species of acid fermentation, already referred to, other acids are developed which decompose the urates and deposit crystals of uric acid. If this takes place after the passage of the urine, of course, it can have no pathological import; but if such changes go on while the urine is still in the passages, the most serious results may follow from the deposition of these crystals, and the formation of gravel and calculi. In such a case as this, the chemical, rational treatment would consist in the administration of alkalis; thus preventing any farther acid fermentation, and at the same time dissolving any of the crystals that may have been deposited. In using these remedies, ammonium salts are to be avoided; because ammonium urate is but little more soluble than free uric acid; salts of potassium and sodium should be used.

If the free uric acid be due to excessive formation, this should be prevented by the administration of oxidizing remedies, which would convert the excess of uric acid into urea. In such cases as this, calcium oxalate will generally be found

deposited with the uric acid, and the treatment should consist of fresh air, exercise of both body and mind, and of acid tonics. Now there is one class of diseases, in which there is excessive formation of uric acid, that cannot be reached by oxidizing agents alone. I refer to those diseases of the heart and lungs which interfere with the circulation, producing venous stasis. In these cases, attention must be given to the organ diseased, and in the meanwhile the deposition of uric acid or urates prevented by the use of alkalis; for no amount of acid tonics could prevent the excessive formation of uric acid, so long as the blood, loaded with carbonic acid and nitrogenous poisons, stagnates in the veins; but as soon as the circulation is normal the proportions of uric acid and urea become natural.

*Urates* are the most common constituents of urinary deposits. They vary in color from white to crimson; the higher the color the more serious are the indications. The nature of the deposit may be determined by the fact that it is cleared up by heat, by the separation of the deposit by decantation or filtration, and the application of the murexid test, or by the addition of nitric acid, and the formation of crystals of free uric acid. The deposition of urates after passage of the urine may be due (1) to deficiency of water; not being enough to hold the urates in solution when the temperature of the body ceases to aid; (2) to excess of urates. Any sudden change of life may cause a temporary deposit of urates; for instance, a person of sedentary habits takes violent exercise and perspires freely; more than the ordinary amount of water passes off through the skin, and leaves a deficiency to be excreted by the kidney; consequently, as soon as the urine cools, urates are deposited in a large quantity, attracting the attention of the person, and if he be ignorant on this subject, often frightening him greatly. It is true that the urine should contain no deposit, and this is an abnormal condition, but one that nature herself will right in a short time. Urates were formerly called critical discharges, and there is one class of affections in which they may be so regarded. I refer to severe febrile affections of an inflammatory nature; in these cases, the sudden appearance of a deposit

of urates is indicative of a change for the better; because they show that so much of the poison has been eliminated. But if there be a frequent deposit of urates, it should not be overlooked nor passed lightly by, but should be studied, and it will be found that everything that I have said with reference to free uric acid applies equally to such a deposit of urates. Since the causes, effects and treatment are the same as given under uric acid, a repetition will be unnecessary.

Hitherto, we have only considered the injurious effects following upon an excess or upon a deposit of urates, but the non-elimination of this substance, and a consequent deficiency in the urine, demands our attention. It is this which leads to gout; the urates are deposited in parts of the body, especially in the joints. A deficient excretion of uric acid can be determined with accuracy only by a quantitative estimation. The elimination of the retained uric acid may be secured by the use of sodium phosphate, potassium bicarbonate, etc., which dissolve and eliminate the uric acid and acid urates. After this, the excessive formation in the body may be prevented by colchicum, quinia, digitalis, etc.

HIPPURIC ACID,  $\text{—C}_9\text{H}_9\text{NO}_3$ .

§ 151. This acid is a constant and important constituent of the urine of the horse, ox, and other herbivorous mammals. It is also found, though in small quantity, in human urine, in which it is increased by the consumption of certain fruits, as plums, and by the administration of benzoic acid, the balsam of Peru, and some other medicinal agents. Hippuric acid has been detected in the scales which form on the skin in ichthyosis.

*Preparation.*—(1) Hippuric acid is best obtained from the urine of the horse or ox as follows: Concentrate from 600 c. c. to 1000 c. c. of the *fresh* urine to a syrup; extract this with alcohol; filter the alcoholic extract and concentrate on the water-bath until all the alcohol is driven off; to the cold residue add cold hydrochloric acid and allow to stand when hippuric acid crystallizes. These crystals may be collected upon a filter and dried by pressure between folds of blotting paper. They may

be purified by solution in boiling water, agitation with animal charcoal, filtration and concentration.

(2) The traces of hippuric acid may be obtained from the urine of man by the following process: Concentrate 1000 c. c. of the urine to a syrup on the water-bath; to this syrup, add alcohol acidified with hydrochloric acid; agitate well and filter. Wash the residue with alcohol, neutralize the united filtrate and washings and heat on the water-bath until all the alcohol is driven off. To the remaining portion, add oxalic acid, then shake with pure ether to which ten per cent. of alcohol has been added. Allow to stand until the ether separates. Shake repeatedly with ether and remove the ether from the united ethereal extract by evaporation. To the residue, add calcium hydrate to a feebly alkaline reaction, warm and filter. Wash the residue on the filter with water which dissolves the calcium hippurate. Concentrate the filtrate and add hydrochloric acid sufficient to produce an acid reaction. Allow to stand when the hippuric acid crystallizes; pour off the supernatant fluid and test the crystals by microscopical examination. Place some of the crystals in a test tube, add a few drops of strong nitric acid and heat to dryness when the peculiar odor of nitrobenzole will be given off if the crystals are hippuric acid.

*Properties.*—Hippuric acid forms in large four-sided prisms which are terminated at each extremity in short pyramids. These crystals are sparingly soluble in cold water and ether, freely soluble in alcohol. Its solubility in alcohol affords an easy means of separating hippuric acid from uric acid. If a urinary deposit is suspected to be composed of, or to contain both uric and hippuric acids, collect it on a filter and wash it with alcohol acidified with hydrochloric acid, when the hippuric acid will be dissolved and may be obtained in the crystalline form on concentrating the filtrate.

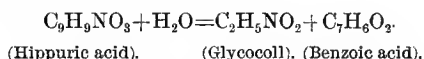
The solution of hippuric acid in hot water has a decidedly acid reaction. This is a monobasic acid and unites with many bases forming characteristic crystalline salts. The hippurate of silver,  $\text{AgC}_6\text{H}_5\text{NO}_2 + \text{H}_2\text{O}$ , is soluble in hot water and is deposited on cooling in fine, glistening needles. If to an aqueous solu-



tion of some hippurate, a few drops of a neutral solution of ferric chloride be added, a brown, amorphous precipitate of the hippurate of iron falls. This precipitate is insoluble in water, soluble in dilute alcohol and in the urine.

In the presence of decomposing matter, hippuric acid is soon changed into benzoic acid; for this reason, all tests for this acid should be made with fresh urine. A disregard of this fact has caused some experimenters to report that hippuric acid is not a constant constituent of the urine of the herbivorous mammals.

If some crystals of dry hippuric acid be heated in a test tube, it will be decomposed; benzoic acid will be given off and deposited upon the upper part of the test tube. Besides benzoic acid, an oily, red fluid with an aromatic odor is obtained by the dry distillation of hippuric acid. If hippuric acid be boiled with nitric acid for some time and the solution then allowed to cool, benzoic acid crystallizes from the solution. The hippuric acid has been decomposed into benzoic acid and glyccoll, the latter remaining combined with the nitric acid after the former has crystallized. The same change is produced by boiling hippuric acid with hydrochloric or sulphuric acid and is represented by the following equation:



If benzoic acid be administered internally, it appears in the urine as hippuric acid. If 15 grains of benzoic acid be taken at bed-time, and the urine passed on rising the next morning be concentrated and acidified with hydrochloric acid, crystals of hippuric acid will soon form and may be recognized by microscopical examination and by their solubility in alcohol.

§ 152. *Physiology*.—Evidently, hippuric acid is formed in the body by the combination of benzoic acid and glyccoll; but the question arises here, what is the source of the benzoic acid necessary to the production of hippuric acid? Do we obtain sufficient benzoic acid as such in our ordinary food to account for the hippuric acid excreted? This question must be answered in the negative, for it has been shown by Weissmann that when

living upon albuminous food only, man continues to excrete hippuric acid. It is true that some fruits contain considerable benzoic acid; thus Ducheck found that after eating greengages, his urine contained a large excess of hippuric acid, and subsequent investigation showed him that this fruit contained benzoic acid in considerable quantity; this has been confirmed by Thudichum. But it has been found that our ordinary foods contain no benzoic acid, and the same was ascertained to be true of the fodder which constituted the food of a cow whose urine contained much hippuric acid. This question as to the source of benzoic acid seems now to be settled by the discovery of Ure, that benzoic acid is produced by the oxidation of albumen. Proust has also obtained oil of bitter almonds and benzoic acid by the action of sulphuric acid and potassium bichromate upon albuminous substances. From this, it is evident that the sources of benzoic acid in the human body are at least two: one constant and furnishing but a small quantity of the acid; the other variable, sometimes furnishing large quantities and at other times being absent. The oxidation of the albuminous constituents of the food, and when food is wanting, of the nitrogenous tissues of the body, furnishes the small but constant supply of benzoic acid from which results some of the hippuric acid daily excreted. From this source, is derived the hippuric acid which has been found to be present in the urine of man after prolonged fasting.

When foods or medicines containing benzoic acid are taken, the hippuric acid is greatly increased. Besides greengages, which have already been referred to, cranberries and blackberries have been found to increase the amount of hippuric acid. Reason as well as experiment would show that there is a limit to the amount of hippuric acid which may be obtained from this source. This limit depends upon the amount of glycocholl available for this purpose in the body; thus Ducheck found that after the administration of 1 gram of benzoic acid, 0.714 of a gram of hippuric acid could be obtained from the urine, which contained no benzoic acid; consequently all of the benzoic acid administered in this case was probably excreted as hippuric

acid. However, the same experimenter found that after the administration of benzoic acid in quantities of 2 grams and over, the amount of hippuric acid remained at about 1.8 grams and did not increase with increased consumption of the benzoic acid; while in these cases, the unchanged benzoic acid could be detected and estimated in the urine. It is but just to state that the experiments of Duchek have been questioned and need confirmation.

Heidenhain\* gives the following reasons for believing that the benzoic acid and glycoll unite in the kidney:

(1) While the urine of herbivora on ordinary grass food is very rich in hippuric acid, this substance has not been found in the blood of the same animals, either while living under normal conditions or after extirpation of the kidneys. In the kidney itself Meissner and Shepard found hippuric acid, while Kochs failed wholly to find it in the kidney of a calf and found it present only in unweighable quantities in the kidneys of oxen.

(2) After the injection of benzoic acid into the stomach of rabbits and dogs, and while the urine was rich in hippuric acid, Meissner and Shepard could not find a trace of it in the blood or any other fluids of the body. After the simultaneous injection of benzoic acid and glycoll into the blood of dogs, Schmiedeberg and Bunge found in the blood under normal conditions a small amount of hippuric acid, after ligating the ureters they found a larger amount, but after ligating the renal blood-vessels, they could find no trace of it.

On the other hand Meissner and Shepard after injecting benzoic acid into the stomach of rabbits and ligating the renal blood-vessels, found both benzoic and hippuric acids in the blood. This has been confirmed by Salomon. This experimenter injected benzoic acid into the stomach of nephrotomized rabbits and found hippuric acid in not insignificant amounts in the liver, muscles and blood. From this it seems that in rabbits there are other tissues, as well as the kidney, in which hippuric acid may be formed.

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\* *Handbuch der Physiologie.*

(3) If oxygenated blood, to which benzoic acid and glycocoll or the former only has been added, be passed through an extirpated kidney of a dog, hippuric acid is formed. If kept in a cool place the kidney is capable of causing this synthesis for 48 hours after extirpation. •

(4) The kidney when cut into large pieces still forms hippuric acid when digested with blood containing benzoic acid and glycocoll, in the presence of oxygen. On being crushed or frozen the kidney cells lose this property.

From what has been said, it will be seen how unwise it is for one to study the excretion of hippuric acid without any regard to the kind and amount of food. What has already been said with regard to the importance of taking into consideration the nature of the ingesta when studying the amount of urine, the reaction, the quantity of urea and uric acid, will apply here and in fact to the study of every constituent of the egesta. The average amount of hippuric acid excreted during 24 hours by a healthy man, living upon ordinary mixed food, is about 8 grains.

Quite a number of experiments have been made by Erdmann and others to show that the amount of hippuric acid excreted by horses varied as the animal was at rest or at work. It was thought that when the animal was at work a part of the carbon of the hippuric acid was converted into carbonic acid and excreted by the lungs; while the remainder of the carbon appeared in the urine as benzoic acid. In confirmation of this theory it was found that the urine of some horses used for plowing contained benzoic acid, but no hippuric acid; while that of other horses kept at rest contained much hippuric acid and no benzoic acid. However, subsequent investigation has shown that the presence of benzoic acid in the urine of the horses used in plowing was due to the decomposition of the hippuric acid after emission, and if the fresh urine of any horse be examined it will be found to contain hippuric acid. Most observers are now united in the opinion that the daily excretion of hippuric acid, like that of urea and uric acid, is not modified by muscular exertion.

§ 153. *Pathology.*—On account of its solubility in water and the small amount generally present, hippuric acid seldom forms a deposit; though such deposits are occasionally observed. It is found in long, needle-shaped crystals, often arranged in groups. They are to be distinguished from uric acid by the fact that the hippuric acid crystals contain but little or no coloring matter, are semi-transparent and are longer than uric acid crystals. But in all cases the deposit should be collected and washed with boiling alcohol. From the alcoholic solution, hippuric acid, if present, re-crystallizes on cooling. Hippuric acid may possibly be mistaken for calcium phosphate, but the former occurs only in urine that is decidedly acid; while the latter is generally found in urine that is feebly acid, neutral or alkaline. But in cases of doubt, a drop of hydrochloric acid should be added to the crystals when, if they are phosphates, they will quickly disappear, while any hippuric acid will remain undissolved. Crystals of the acid phosphate of calcium occur in urine that is strongly acid, but the solubility of the phosphate in hydrochloric acid distinguishes it from hippuric acid.

#### PHOSPHATES.

§ 154. In the urine, phosphorus exists as phosphoric acid combined with the bases calcium, magnesium, sodium and potassium. Accordingly, there are alkaline and earthy phosphates; calcium and magnesium phosphates belonging to the latter class, while sodium and potassium phosphates constitute the former. In normal urine, the excretion of alkaline phosphates is much greater than that of the earthy; for the simple reason that alkaline bases are more abundant in our food and blood than earthy bases. However, the exact proportion between the quantities of these two kinds daily excreted varies greatly with our food.

Of the earthy phosphates, the magnesian is the more abundant in the urine of growing children, because a greater part of the calcium is taken up in the growth of the bones and teeth; while the urine of old people contains more calcium than magnesium phosphate (Harley). If normal urine be rendered alkaline by the addition of ammonium hydrate, the earthy phosphates are precipitated. The magnesium phosphate com-

bines with the ammonium and is deposited as ammonio-magnesium or triple phosphate, in pennate or stellate crystals; while the calcium phosphate is simply thrown out of solution and forms a granular deposit. If instead of adding ammonium hydrate directly to the specimen, the urine be allowed to stand until the urea gradually decomposes, with the slow formation of ammonium carbonate, the ammonio-magnesian phosphate crystals will be prismatic and not stellate or pennate. Thus, if the urine has undergone decomposition in the bladder, microscopical examination will reveal prismatic forms of the triple phosphate. Ammonio-magnesium phosphate is never deposited in urine which is not ammoniacal; consequently, if the specimen under examination be acid, or be alkaline from a fixed alkali, or if the odor of ammonia be not perceptible, the presence of these crystals need not be suspected. Of these forms, the prismatic only is to be regarded as of pathological import; for the presence of the stellate or pennate crystals indicates the accidental or intentional addition of ammonium to the urine after its passage, and consequently does not in any way show an abnormal state of the patient. Whether the prismatic form be indicative of any condition affecting the health of the individual depends upon the time elapsing between the passage and examination of the specimen. If these crystals are formed in the bladder, the urine will be ammoniacal when emitted and will immediately deposit a sediment which will be found on microscopical examination to contain the prismatic triple phosphates. This is the case when there is excessive irritation of any part of the urinary tract and generally arises from retention; in such a case the retained urine becomes ammoniacal and increases the irritation, while the mucus which is poured out hastens the decomposition of the urea.

On the other hand, if perfectly normal urine be set aside after its emission, it will sooner or later become alkaline and deposit triple phosphates; consequently it is necessary that the analyst know whether decomposition took place within the body or after emission, before he is ready to announce the pathological indications of his examination.

*Ammonio-Magnesium Phosphate.*—All forms of triple phosphates are beautiful microscopic objects. The stellate and penate crystals are sometimes composed of large-interlacing rods; while at other times minute and delicate fringing borders the larger branches. The prismatic crystals are not unfrequently large enough to be visible to the unaided eye, and form beautiful polariscopic objects. They consist of various modified prisms with many truncations. Not unfrequently the careless student finds these crystals of a deep blue color; a drop of acid touches some part of the microscope and dissolves a little of the copper and this solution stains the crystals. If the prisms be very short, they may be mistaken for octohedrons of calcium oxalate; consequently, the microscopic examination should be confirmed by the chemical test. If the crystal be a triple phosphate, it will disappear upon the addition of acetic acid; while if it be an oxalate, it will be insoluble in acetic, soluble in hydrochloric acid. Again kreatinin may be mistaken for prismatic phosphates; but kreatinin is generally found in acid urine.

*Calcium Phosphates.*—In the urine there are at least two phosphates of calcium and it is a matter of no little importance as to which forms a deposit. The neutral phosphate of calcium  $\text{Ca}_3(\text{PO}_4)_2$ , may occur as a sediment in urine that is either feebly acid, neutral or alkaline. It never occurs in a crystalline form, but always as a granular mass. When urine containing a sediment of this salt is agitated, the deposit is easily distributed and floats through the fluid as a cloud of mucus, from which the phosphate is distinguished by its ready solubility in acids. When it is deposited in feebly acid urine, this phosphate may be mixed with crystals of uric acid and consequently may be mistaken for urates, but the phosphate does not dissolve on the application of heat and is thus distinguished from urates. In neutral urine and in that alkaline from a fixed alkali, the neutral phosphate of calcium is mixed with magnesium phosphate. Whether a deposit of amorphous phosphates contains this salt of calcium or consists wholly of magnesium phosphate may be decided by the following process: Collect the deposit upon a filter, wash it with a little water and dissolve in hot acetic acid.

To this solution add ammonium oxalate when any calcium, which may be present, will be precipitated as an oxalate. After the removal of the precipitated calcium oxalate by filtration, magnesium may be precipitated from the filtrate on the addition of ammonium hydrate. In ammoniacal urine, this substance is mixed with crystals of ammonio-magnesium phosphate.

The neutral phosphate of calcium is less soluble in hot urine than it is in cold urine; for this reason, it often happens that the application of heat to a specimen of urine which is clear, yet but feebly acid, causes a turbidity. The precipitate thus formed is distinguished from albumen by its solubility in nitric acid. It is this phosphate which gives the cloudy appearance to urine which is passed soon after eating. Mixed with magnesium phosphate, it may be thrown down from normal urine, on the addition of sodium or potassium hydrate.

*Acid Phosphate of Calcium,  $\text{CaHPO}_4$ .*—This substance is occasionally found as a urinary deposit. It occurs only in urine that is decidedly acid and it invariably appears in a crystalline form. These crystals may be needle-shaped, prismatic, or rhombic tablets. The needle-shaped variety is distinguished from uric acid by the absence of coloring matter and from both uric and hippuric acids by the ready solubility of the phosphate in dilute hydrochloric acid. The prismatic form cannot be distinguished by optical examination from the corresponding form of the triple phosphates; but the triple phosphate occurs as a deposit only in ammoniacal urine, while this form of calcium phosphate is deposited only in acid urine.

Calcium phosphate may be obtained artificially in a crystalline state by dissolving some of the amorphous deposit to saturation in acetic acid and allowing the acid solution to stand for some days, when crystals will be deposited. Again, if a solution of sodium phosphate be treated with one of calcium chloride, an amorphous precipitate of calcium phosphate is thrown down. After this has stood for some days, microscopical examination will reveal minute, thin, rhombic plates.

*Alkaline Phosphates.*—On account of their free solubility in



both acid and alkaline urines, these phosphates never form a spontaneous deposit. They may be separated from the earthy phosphates by rendering the urine alkaline and then filtering. The earthy phosphates, being insoluble in alkaline urine, are thus removed. From the filtrate the phosphoric acid, which is combined with sodium and potassium, may be precipitated as ammonio-magnesium phosphate on the addition of ammonium hydrate and magnesium sulphate.

*Sodium Phosphate,  $\text{Na}_2\text{HPO}_4$ .*—This salt may be obtained from the urine by the following method: Concentrate some urine to a syrup on the water-bath; allow to stand for some time when many of the urinary salts will be deposited in a crystalline mass. Pour off the supernatant syrupy fluid into a clean, small beaker, and add to it alcohol in excess. Sodium phosphate is precipitated by the alcohol, and after standing for some time may be obtained for microscopical examination. It appears in colorless, transparent, rhombic prisms, which are freely soluble in water. This salt is identical with the ordinary sodium phosphate of the pharmacopœia.

*Acid Phosphate of Sodium,  $\text{NaH}_2\text{PO}_4$ .*—This salt may be prepared artificially by boiling uric acid with a solution of the ordinary sodium phosphate when the uric acid takes up one equivalent of the sodium and is converted into a urate. The acid phosphate exists in the syrupy fluid from which the ordinary phosphate is obtained; but it crystallizes more slowly after the addition of the alcohol, and may be found in the sediment after the mixture has stood for three or four days; or its deposition may be hastened by the addition of ether to the mixture of alcohol and the syrupy fluid. It forms in a variety of shapes, the most common of which is the rhombic prism. The acid phosphate of potassium,  $\text{KH}_2\text{PO}_4$ , is found in the urine in very small quantity, and resembles the corresponding salt of sodium.

§ 155. *Physiology of Phosphoric Acid.*—The food of man contains large quantities of phosphorus, as phosphoric acid combined with various bases, and smaller quantities existing in complex organic compounds, as albumen. One pound of

beef contains on an average about 60 grains of phosphates; while an equal weight of bread contains as much as 65 grains. Moreover, potatoes and other articles of food are rich in phosphates. Albumen contains phosphorus, and this, during its passage through the body, is oxidized, and appears in the urine as phosphoric acid combined with bases. In the growing child, much of the phosphorus of the food is used in the construction of various tissues; as bone, muscle and brain contain this element. But in the adult the amount of phosphorus needed for the repair of tissue is probably very small, and the amount excreted varies with the food. If no food is taken, then the tissues of the body are consumed in the production of force necessary to the maintenance of life, and consequently phosphates continue to appear in the urine. But from what has been said, it is evident that when no food is taken, the excretion of phosphorus falls to a minimum, unless there be some disease which causes undue disintegration or oxidation of tissue.

The exact physiological office of the phosphorus, which is present in the food as inorganic matter, is not known. Evidently it cannot be a direct source of force from changes within itself; for it has already reached the limit of oxidation and must return to the plant in order to receive a new supply of force, or to be deoxidized. No doubt our ordinary food contains more of this inorganic material than is absolutely necessary; but that some of it is essential to the healthy activity of the body is a fact proven beyond dispute. It is probable that the inorganic salts of phosphorus are of value, and are even necessary in the food of the adult, principally on account of their influence over other substances; thus, in the blood, the acid phosphates hold certain substances in solution and prevent the formation of others. That the urine contains some phosphorus in organic combination may be proved by estimating the amount of phosphoric acid contained in a given volume of urine, and then evaporating another equal portion of the same urine to dryness, burning the residue until all the organic matter is destroyed, dissolving the ash in water acidified with acetic

acid, and estimating the amount of phosphoric acid in this solution. It will be found that the quantity has been increased by burning. The phosphorus of the organic matter has been converted into phosphoric acid. The exact nature of the organic compound, which contains phosphorus and is constantly present in the urine, is not known. Both lecithin and glycerin-phosphoric acid have been found in the urine, but these observations have not been sufficiently numerous to enable us to decide whether one or both of these are constant constituents of the urine or not,

The quantity of phosphoric acid contained in the 24 hours urine is very variable, even in a state of perfect health. These variations depend upon the kind and amount of food and probably to some extent upon the time of day at which the meals are taken. From a few experiments made upon this point, it seems that when late dinners are taken, less of the phosphates and indeed of all the constituents of the food is absorbed; while the fæces are so much richer in this constituent and increased in total amount. A healthy man living upon ordinary mixed food will excrete from 2 to 5 grams of phosphoric acid (estimated as  $P_2O_5$ ) during 24 hours. Drinking much water increases the excretion of phosphoric acid. This is probably due to, at least, two causes: (1) a greater proportion of phosphates are held in solution in the intestines and consequently a correspondingly great quantity is absorbed; (2) the water increases arterial pressure and consequently augments the quantity of urine and urinary salts.

§ 156. *Pathology.*—The study of the pathology of phosphates divides itself into two distinct parts. These are, (1) deposition of phosphates in the urinary passages; (2) an excessive excretion of phosphoric acid. Both of these conditions may exist at the same time, but they are not necessarily dependent upon each other, and indeed most frequently exist separately. Phosphates may be deposited even when they are excreted in abnormally small quantities; the deposition depends upon the reaction of the urine and not directly upon the amount of phosphates present. Again there may be three times as many phosphates

present as there should be, and still the urine be perfectly clear and contain no deposit. The pathological conditions indicated by a deposition of phosphates and those indicated by an excessive excretion are wholly different. The presence of phosphates in a deposit is to be ascertained by microscopical and chemical examination; while the presence of an excess of phosphoric acid can be ascertained only by a quantitative examination of the 24 hours urine.

(a) *Deposition of Phosphates.*—Only the earthy phosphates are ever deposited spontaneously in the urine, and when such a deposit occurs, it is important to decide as to the exact nature of the phosphates which form the deposit. The deposition of ammonio-magnesium phosphate in the urinary passages is always due to some local cause. This form of phosphates may be deposited either in the kidney or in the bladder and is a frequent constituent of both renal and vesical calculi. The treatment must be directed to the local cause; thus if there be retention of urine, this must be relieved; if there be undue irritation of any part of the urinary tract, from which an excess of mucus is poured out, the irritation must be relieved.\*

The normal phosphate of calcium is found deposited in the urine with the triple phosphates, in the cases mentioned above; but in other instances, the deposit will be found to consist wholly of the amorphous phosphates of calcium and magnesium. This deposit is due to an excess of fixed alkali, or to an excess of the earthy phosphates themselves. If the urine be alkaline from a fixed alkali, the cause is to be sought, either in the food, or in an excess of alkalis in the blood, or in a low state of vitality.† The acid phosphate of calcium sometimes occurs as a urinary deposit and may form calculi. The treatment should consist of exercise in the open air and the administration of the weak mineral acids, as carbonic and phosphoric, and of organic acids, as acetic (in old cider) and benzoic.

(b) *An Excess or Deficiency of Phosphates.*—In diseases of the stomach, as in gastric catarrh, the excretion of phosphates in

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\* For details in regard to ammoniacal urine, see p. 200 et. seq.

† See p. 199 et. seq.

the urine is diminished; while the fæces contain an excess of this constituent. If lactic or butyric acid replace the hydrochloric acid of the gastric juice, less of the mineral ingredients of the food will be dissolved and absorbed; while if acids are wholly wanting in the secretion of the stomach, still smaller quantities of the phosphates will reach the blood. In structural diseases of the kidneys, the amount of phosphoric acid in the urine is often very small and the quantity is not increased by diuretics in proportion to the increase in the water. In diseases which interfere with the free action of the respiratory organs, the excretion of phosphorus as phosphoric acid is sensibly diminished. This is due to the fact that the oxidation of the albuminous constituents of the food is imperfect and the phosphorus from this source is not converted into phosphoric acid. In these diseases, the amount of phosphorus excreted in organic combination is probably increased, but experimentation is needed on this point. In acute febrile diseases, the amount of phosphoric acid has been found to be much less than normal; but this is probably due to the low diet.

In inflammatory diseases of the nervous system, the patient, although living upon a low diet, excretes more phosphoric acid than in health. This probably arises from the disintegration of the nervous tissue and the oxidation of the phosphorus of the lecithin. Harley found that a man, who had received an injury in the neighborhood of the fourth ventricle (as diagnosed from the presence of sugar in the urine), eliminated 8.749 grams of phosphoric acid; "and this, too, at a time when he was taking very little food, and that little poor in phosphates."

It has been shown (p. 151) that in rickets, osteomalacia and some other diseased conditions, the bones contain but a small proportion of inorganic matter. Now during the progress of these diseases, the urine contains an excess of phosphoric acid. This essential constituent of the osseous system is unduly removed and washed out of the body with the urine. It is probable that the solution of the mineral constituents of the bones is affected by the abnormal development of some acid. If we knew the nature of this acid and the cause of its devel-

opment, the rational treatment of these diseases would probably be found; but as it is, the physician can do no better than to see that his patient is properly fed and clothed, has plenty of fresh air and good water, and to administer phosphates to supply the place of these removed, and iron, quinia and strychnia to tone up the system.

#### SULPHATES.

§ 157. The greater part of the sulphuric acid contained in normal urine is combined with potassium, while traces of the sulphates of sodium and calcium are occasionally detected. Of these, the calcium salt is the only one that ever occurs in deposit and it is rarely met with. Calcium sulphate crystallizes in long, needle-shaped crystals which are much finer than those of hippuric acid, and resemble tyrosin; from the latter, the calcium salt is distinguished by the ready solubility of the tyrosin in ammonium hydrate. Crystals of calcium sulphate are not unfrequently observed in the urine of the horse; and they may be obtained in abundance by giving the horse magnesium sulphate in his drink, collecting the urine passed by the animal afterwards, acidifying and allowing it to stand, when crystals of gypsum will form.

Since the sulphates in urine are in solution, the appearance of the specimen affords no evidence as to whether this constituent is in excess or not. If any urine be acidified with hydrochloric acid and then treated with a solution of barium chloride, barium sulphate is precipitated and will be found insoluble in acids.

§ 158. *Physiology*.—If sulphur be taken into the body as soluble sulphates, free sulphur, or in organic combination, it increases the amount of sulphates in the urine. Our food contains alkaline sulphates and organic compounds as albumen, which furnish some sulphur. The first of these passes through the body unchanged, with this exception, that the sodium sulphate is converted into the corresponding salt of potassium; thus, if sodium sulphate and potassium chloride be taken into the body, a mutual exchange takes place and the sulphuric acid is excreted as a potassium salt and the chlorine appears

in the urine as sodium chloride. The other source of the sulphuric acid of the urine is in the oxidation of the sulphur of certain organic constituents of the food and of the body. That all of this sulphur is not completely oxidized when excreted in the urine may be proved in the following way: To 500 c. c. of normal urine, add sufficient hydrochloric acid to render it strongly acid and then remove all the sulphates by precipitation with barium chloride and filtration. To be sure that all the sulphuric acid has been removed, add a little more barium chloride to the filtrate and if no precipitate forms, all the sulphates have been removed. Now treat the filtrate for several hours with a current of chlorine gas. Soon a white precipitate will be observed to fall. The chlorine has oxidized the sulphur which had existed in organic combination, and as fast as this is oxidized to sulphuric acid it precipitates the excess of barium chloride in the solution and falls as barium sulphate. After all the organic matter has been destroyed by the chlorine the precipitated sulphate may be collected upon a weighed filter, dried and weighed.

While the amount of sulphuric acid excreted in a given time depends largely upon the food, this constituent does not entirely disappear from the urine of one who abstains from food. The small quantity, which continues to be present, is due to the oxidation of the tissues of the body. Anything, which improves oxidation, increases the excretion of sulphates; for this reason, fresh air and nitro-muriatic acid increase the quantity of this urinary constituent. The average amount of sulphuric acid excreted as such in the 24-hours' urine is about 2.2 grams, while the unoxidized sulphur furnishes about .2 gram more. If a person is inactive and breathes impure air, much of the sulphur will be excreted in organic compounds; while on the other hand, if he exercises body and mind as he should, and obtains sufficient pure air, the greater part of these organic compounds will be changed into inorganic matter. Finally, to condense and conclude, it may be said that the amount of sulphuric acid excreted in a given time depends, (1) upon the food, and (2) upon the conditions under which the food passes through the body.

§ 159. *Pathology.*—Sulphuric acid bears the same relation to the unoxidized sulphur of the food and tissues as urea bears to the less highly oxidized nitrogen of the same food and tissues; consequently it is found that sulphur and urea are decreased or increased by the same causes. In cases of indigestion, the sulphuric acid of the urine is diminished; the same is true in cholera, and for the reason given for the diminished formation of urea in this disease. It is not simply the small amount of sulphuric acid that may be present in the urine that is to be regarded as of pathological import; but it is the corresponding increase in the quantity of sulphur that leaves the body in organic combination. All escape of unoxidized food from the body is so much lost in the production of force. When an excess of nitrogen leaves the body as uric acid, or an excess of carbon as oxalic acid, or an excess of sulphur as cystin and other organic substances, then so much latent force escapes conversion into manifest force. Of course, the body of man is not so perfect that it can obtain and utilize all the force which exists in the latent condition in the food; but it is conducive to health to secure the normal degree of oxidation.

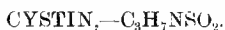
In skin diseases, the amounts of both urea and sulphuric acid are decreased; while uric acid and unoxidized sulphur are correspondingly increased. In the same diseases, calcium oxalate is generally found in large quantities in the urine. It is a question as to whether these products of an imperfect oxidation cause the diseased condition of the skin, by poisoning the blood, or whether the impairment of the function of the skin is the cause of the arrest in oxidation. It is probable that these conditions, after being inaugurated, mutually react upon each other, and that treatment appropriate for each should be used at the same time.

When there is a deficiency of sulphuric acid and an excess of unoxidized sulphur in the urine, the latter should be converted into the former. This can be done by the use of nitromuriatic acid and exercise in the fresh air. The acid acts here, as in cases of an excess of uric acid, by virtue of its oxidizing properties, especially in improving digestion, and not because it is an acid.



In fevers, the tissues of the body are consumed or burnt, and sulphuric acid is formed, just as it would be by oxidizing sulphur, and is found in the urine in increased quantity. In diabetes, the sulphuric acid is increased in proportion with and for the same reason that urea is augmented in that disease.

As has been stated, calcium sulphate is sometimes found in deposit; but it is not known ever to be present in the urine of man in quantities sufficient to produce pathological results. In the herbivora, this salt sometimes forms calculi. Beale observed crystals of gypsum in the uriniferous tubules.



§ 160. *Occurrence.*—This is the only one of the well-known organic constituents of the urine which contains sulphur. It is sometimes found as the sole or principal constituent of urinary calculi of men and of dogs. At other times it may be detected in urinary deposits or in solution in the urine.

*Preparation and Identification.*—Cystin is purified by dissolving the stone in ammonium hydrate, filtering and allowing the filtrate to evaporate spontaneously, when the cystin forms in colorless, six-sided plates. These are distinguished from uric acid crystals of the same form by the absence of color in the cystin crystals and their ready solubility in ammonium hydrate. From acid solutions, cystin is precipitated by the addition of ammonium carbonate; and from alkaline solutions, by acetic acid. Cystin is insoluble in water, alcohol and ether; soluble in ammonium hydrate, fixed alkalis and carbonates of sodium and potassium, but insoluble in ammonium carbonate. It is soluble in the mineral acids and in oxalic acid, but insoluble in tartaric and acetic acids. If a solution of cystin in sodium or potassium hydrate be boiled, the cystin is decomposed with the formation of an alkaline sulphide, ammonia, and an inflammable gas. With the mineral acids cystin forms crystalline salts, which are easily decomposed.

*Tests.*—If some cystin be placed upon a piece of silver, then moistened with a drop of a solution of sodium hydrate

and heated, the silver will be stained brown. An alkaline sulphide has been formed, and in turn, this acted upon the silver producing the sulphide of silver. Again if a solution of cystin in sodium hydrate be boiled in a test tube with lead acetate, a black precipitate of lead sulphide will be formed.

Mueller dissolves cystin in potassium hydrate, dilutes the solution with water and then adds some potassium nitroprusside, when, if cystin be present, a beautiful violet color is produced. He holds that this is a more delicate test than any other.

*Detection in Urine.*—It must be remembered that cystin may be present in solution in the urine; indeed I have found it frequently in the urine, but never in deposit. The urine is frequently neutral or slightly alkaline, and often contains traces of pus, showing some irritation. To such urine as this, acetic acid should be added as long as a precipitate is formed. This precipitate, which is amorphous, should be collected on a filter, washed with a little water and then dissolved in ammonium hydrate. The ammoniacal solution should be evaporated gently on the water-bath, when the characteristic crystals of cystin will be obtained. If the urine should be acid, ammonium carbonate should be added and the precipitated cystin mixed with phosphates should be collected, washed and dissolved in ammonium hydrate as before. The phosphates being insoluble in ammonium hydrate will remain upon the filter.

Fresh urine containing cystin has a sweet-briar odor; while after decomposition sets in, hydrosulphuric acid gas is given off, and may be recognized by its odor and by blackening silver.

§ 161. *Physiology.*—Cystin is probably an intermediate stage in the formation of sulphuric acid by the oxidation of the sulphur of the food and tissues. The liver is supposed to have some influence over the formation of cystin, and it is probable that it results from the splitting up of the albuminous constituents of the food. In some diseased states, the liver on post-mortem microscopical examination has been found to contain crystals of cystin. Cystin resembles taurin in the per cent. of

sulphur which it contains, and may result from failure to oxidize the sulphur of the taurin. At present only conjectures can be offered with regard to the physiology of this substance, as all positive knowledge on this point is wanting.

§ 162. *Pathology.* The condition, which is represented by the presence of cystin in the urine, is known as cystinuria. This is not so rare as is generally supposed. But few cases are reported for the reason that a person may excrete cystin in his urine for years; and suffer from no particular pains which would call attention to the urinary organs; then again, comparatively few physicians in general practice ever suspect and test for cystin. Within the past three years I have met with two cases of cystinuria. The first was a lady, 30 years of age, unmarried, and who complained of dull headaches, probably due to indigestion, and also of slight irritation of the bladder. The daily excretion of urine was found to be normal in quantity, but was slightly ammoniacal, and after standing for some hours formed a dirty white deposit, which consisted of mucus, phosphates and pus. It was by a mere accident that I was led to suspect cystin. The urine had been examined frequently, and a small bottle full, closely corked, had been standing upon my table for several days awaiting examination. One day, I happened to observe the bottle, and took it up, thinking that I would throw it out and obtain a fresh specimen for examination. I removed the cork and observed immediately a strong odor of hydrosulphuric acid gas; while a silver watch placed over the mouth of the bottle was soon blackened. The addition of acetic acid threw down a slight flocculent precipitate, which was collected upon a filter and dissolved in ammonium hydrate. The ammoniacal solution was gently evaporated in a watch-crystal on the water-bath, and the residue examined under the microscope, when beautiful six-sided plates of cystin were observed. This residue was then further tested by dissolving it in potassium hydrate and boiling this solution with some lead acetate when lead sulphide was formed. After this several analyses of the twenty-four hours' urine were made, but the quantity of cystin was not estimated. The following expresses an average analysis:

Total quantity for the 24 hours=1440 c. c.  
Deposit, slight and of a yellow color.  
Color of the fluid, yellow.  
Odor, of sweet briar.  
Reaction, alkaline from ammonia.  
Specific gravity, 1011.  
Crystals of ammonio-magnesium phosphate.  
Pus, present in small quantity.  
Phosphoric acid=2.60 grams.  
Urea =14.48 grams.  
Sulphuric acid = 1.38 grams.  
Chlorides = 7.20 grams.  
Albumen, a trace, and due to pus.

It is seen from this that both the urea and sulphuric acid are present in small quantity. The uric acid was not estimated. The cystin disappeared from the urine when the patient took nitro-muriatic acid and abstained from food containing much sulphur, as beef and eggs. Also the pus disappeared from the urine after this treatment had been followed for some time. However, both the cystin and pus returned as soon as the patient began to eat meat. It is probable that in this case, the cystin was deposited in the bladder, causing some irritation, and the pus, which was poured out, caused decomposition of the urea.

The second case was that of a little boy of 8 years of age. He was anæmic, and had been troubled with sick-headache and dizziness. It was found on inquiry that he was very fond of eggs, and ate largely of them. Cystin was precipitated and detected as in the preceding case. He was requested to abstain from his favorite food, and he was given two drops of the strong nitro-muriatic acid in a tumbler half full of water, after each meal. The medicine was continued for a month, and although two years have elapsed, the symptoms have not returned.

In neither of these cases, was there any evidence of the disease being hereditary. On the other hand, several cases have been reported in which different members of the same family were subject to deposits of cystin. The greatest danger in cystinuria is of the formation of a stone. It is true that as long as so much sulphur is leaving the body without undergoing the

process of oxidation, the person cannot be in the enjoyment of perfect health.

The predisposing causes to this disease are excessive use of foods containing sulphur, want of fresh air and proper exercise; to these, some would add a hereditary disposition; this no doubt has its influence in this as well as in other diseases, but it is more probable that different members of the same family often have this disease because they live in the same atmosphere; they partake of the same kind of food and breathe the same kind of air.

#### SODIUM CHLORIDE,—NaCl.

§ 163. This compound is abundantly distributed in nature, being found in large deposits, and in the water and air. It has been proved experimentally that animals entirely deprived of this article of food do not thrive so well as those which are supplied with it in due quantity. But however essential common salt may be to the healthy condition of man, the majority of people take in their food more of this constituent than is absolutely necessary; this is shown by the large quantity of sodium chloride that is daily excreted in the urine.

*Test.*—To some normal urine in a test tube, add nitric acid sufficient to produce a decidedly acid reaction, then add a few drops of silver nitrate. A voluminous, white precipitate of silver chloride falls, and upon boiling the mixture, this precipitate forms a clot and soon subsides on cooling. Pour off the supernatant fluid, and boil the clot with nitric acid in which the precipitate will be found to be insoluble. Pour off the nitric acid and shake the clot with ammonium hydrate, when solution takes place. The silver chloride is soluble in ammonium hydrate and insoluble in nitric acid. In making this test, it is quite necessary that the urine be poured off from the precipitated silver chloride; for, if this is not done, on the addition of ammonium hydrate, the silver chloride will be dissolved; but at the same time the earthy phosphates will be thrown down, and the novice will think that the chloride does not dissolve.

*Preparation.*—If a solution of sodium chloride in pure water be concentrated, this salt forms in cubes; but in the presence of

urea and some other organic substances, sodium chloride crystallizes in octohedrons; consequently from the urine it always appears in the latter form. These crystals should be obtained from the urine and examined with care; for not unfrequently the beginner places a drop of urine on a glass slide and begins his microscopical examination, soon the water evaporates and large colorless octohedrons form and are mistaken for calcium oxalate. These crystals may be obtained in quantity from the urine by the following process: Concentrate from 200 c. c. to 500 c. c. of normal urine to one-sixth its volume, filter and continue the concentration of the filtrate on the water-bath until a syrupy fluid is obtained; set this aside for 24 hours, when a mass of octohedral crystals of sodium chloride will be deposited. These crystals are mixed with urea and the phosphates of sodium and potassium; from these impurities, the sodium chloride may be freed by the following method: Collect the crystalline mass and press between folds of blotting paper, then place in a crucible and ignite until all the organic matter is destroyed; dissolve the ash in water, boil the solution with animal charcoal and filter. Concentrate the filtrate to a small volume on the water-bath and allow to stand, when sodium chloride crystallizes; while the alkaline phosphates remain in solution in the supernatant fluid, and may be poured off.

§ 164. *Physiology*.—Sodium chloride plays an important part in the animal system. According to Liebig, it influences the development of cells and probably assists in their preservation. The amount of this constituent excreted daily in the urine is less than that taken in with the food. Some of it escapes with the fæces, and some in the perspiration; moreover, some of the chlorine is used in the production of the gastric juice, while a part of the sodium is taken to the liver, and there becomes the base of the glycocholates and taurocholates. In the blood, the presence of sodium chloride assists in holding the albumen in solution and influences the shape of the blood corpuscles. In muscle, bone, and brain, this salt is present not only as common salt, but in organic combination with other substances; thus, if some finely divided muscle be thrown upon a

filter and washed until the filtrate no longer contains chlorides, and then the muscle be transferred to a crucible and burnt, the ash will be found to contain common salt.

If a large quantity of salt be injected into the veins of an animal, it is rapidly eliminated in the urine and perspiration, while it is also increased in the saliva. The rapidity with which this substance is excreted after it has been taken with the food, depends upon the time of day at which it is taken. Like urea and in fact all other constituents of the urine, more salt is excreted during the hours of the forenoon than during the same number of hours later in the day. If large quantities of salty water be drank at night, there is no marked increase in the amount of salt excreted until the next morning. Evidently every experiment upon this subject goes to prove that the processes of life are carried on with greatest vigor during the hours when we are under the influence of sunlight.

It is an established fact that in health, the increased consumption of salt increases for a while the excretion of urea. This is probably due to the stimulating action of salt upon the kidneys. An interesting example of this seems to be furnished in cases of diabetes insipidus when pneumonia supervenes. During pulmonary hepatization and when chlorides are absent from the urine, the amount of the urine often becomes normal. In a case of diabetes insipidus under my observation for a long while, the daily excretion of urine fell, during the stage of pulmonary hepatization of an intercurrent attack of pneumonia, from 12000 c. c. to 1500 c. c. As soon as the chlorides began to reappear, the quantity of urine began to increase, and after the patient recovered, she again excreted daily about 12000 c. c. of urine. A similar case is mentioned by Senator.\* We would hardly be justified in saying that in these cases, the return of the urine to the normal quantity was due to the arrested excretion of chlorides; but it is an interesting fact that if typhus fever (Pribram), acute rheumatism (Dickinson), erysipelas (Senator), pneumonia (Senator and myself), supervene in diabetes insipidus, there is a diminution of the amount of urine

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\* Ziemssen's Cyclopædia, Vol. XVI, p. 1081.

excreted. Now in these same diseases, typhus fever (Parkes), acute rheumatism (Folwarczny), erysipelas (Parkes), pneumonia (first observed by Heller and Redtenbacher), common salt is diminished and frequently is not found at all in the urine. Another fact of interest in this connection is that in diabetes insipidus the amount of chlorides is excessive; thus Vogel found that in a case of this disease as much as 48 grams of sodium chloride were excreted in the 24 hours' urine.

It is evident that the quantity of sodium chloride excreted in the 24 hours urine in health is very variable. According to my experiments, the daily excretion of salt may vary from 5 to 15 grams, the average being about 6 grams. It must be remembered that these figures refer to the quantity of sodium chloride and not to that of chlorine. In cases of starvation, chlorides wholly disappear from the urine, the system refusing to yield that contained in the tissues until more is furnished.

§ 165. *Pathology.*—In certain inflammatory diseases, as pneumonia, the common salt in the urine is diminished and frequently this urinary constituent is absent. This decrease occurs even when the patient consumes much salt in his food. It was formerly supposed that the chlorides were retained in the inflamed lung; but the retention of chlorides is not a condition wholly peculiar to pneumonia, but exists in all acute febrile diseases. They have been found deficient and absent in phthisis, typhus and typhoid fevers, erysipelas, acute rheumatism and cholera. Beale found that when chlorides were absent from the urine in pneumonia they were abundant in the sputa; and the other excretions in the above mentioned diseases should be examined. In all these diseases, the diminished excretion of salt is an unfavorable symptom; while the subsequent increase or the reappearance, after having been absent, is an indication of improvement, and often this is the first evidence of a change for the better furnished the physician. An increased excretion of common salt is not known to be by itself indicative of any pathological condition, but to depend wholly upon the food. The excessive excretion of salt in diabetes insipidus has already been referred to; but this has not been sufficiently



studied to enable us to draw any general conclusions. To conclude, we may say that so far as this constituent is concerned, the urine is abnormal when it contains in the 24 hours' excretion, less than one gram of sodium chloride.

It must be remembered that since common salt is soluble in both acid and alkaline urine, it never forms a spontaneous deposit, but is to be tested for, in the solution, with silver nitrate as already given.

#### OXALIC ACID,— $\text{H}_2\text{C}_2\text{O}_4$ .

§ 166: *Formation*.—Oxalic acid may be produced by the imperfect oxidation of many organic substances; thus, if one part of sugar be boiled with six parts of nitric acid of specific gravity 1.3, as long as red vapors of the oxides of nitrogen are given off, and the solution then be evaporated on the water-bath, oxalic acid will remain in a crystalline form. In the body, it may result from the partial oxidation of many substances; while if the process of oxidation was completed, carbonic acid would be produced. Some of these changes are represented in the following equations:

##### INCOMPLETE OXIDATION.

- (1)  $\text{C}_5\text{H}_4\text{N}_4\text{O}_3 + 3\text{H}_2\text{O} + 2\text{O} = \text{H}_2\text{C}_2\text{O}_4 + 2\text{CH}_4\text{N}_2\text{O} + \text{CO}_2$ .  
(Uric acid). (Oxalic acid). (Urea).
- (2)  $\text{C}_4\text{H}_2\text{N}_2\text{O}_4 + 2\text{H}_2\text{O} + \text{O} = \text{H}_2\text{C}_2\text{O}_4 + \text{CH}_4\text{N}_2\text{O} + \text{CO}_2$ .  
(Alloxan).
- (3)  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3 + 2\text{H}_2\text{O} + \text{O} = \text{H}_2\text{C}_2\text{O}_4 + 2\text{CH}_4\text{N}_2\text{O}$ .  
(Allantoin).
- (4)  $2\text{C}_{57}\text{H}_{110}\text{O}_6 + 216\text{O} = 55\text{H}_2\text{C}_2\text{O}_4 + 4\text{CO}_2$ .  
(Stearin).
- (5)  $\text{C}_6\text{H}_{10}\text{O}_5 + 9\text{O} = 3\text{H}_2\text{C}_2\text{O}_4 + 2\text{H}_2\text{O}$ .  
(Glycogen).

##### COMPLETE OXIDATION.

- (1)  $\text{C}_5\text{H}_4\text{N}_4\text{O}_3 + 2\text{H}_2\text{O} + 3\text{O} = 2\text{CH}_4\text{N}_2\text{O} + \text{CO}_2$ .
- (2)  $\text{C}_4\text{H}_2\text{N}_2\text{O}_4 + \text{H}_2\text{O} + 2\text{O} = \text{CH}_4\text{N}_2\text{O} + 3\text{CO}_2$ .
- (3)  $\text{C}_4\text{H}_6\text{N}_4\text{O}_3 + 2\text{O} = 2\text{CH}_4\text{N}_2\text{O} + 2\text{CO}_2 + \text{H}_2\text{O}$ .
- (4)  $2\text{C}_{57}\text{H}_{110}\text{O}_6 + 326\text{O} = 114\text{CO}_2 + 110\text{H}_2\text{O}$ .
- (5)  $\text{C}_6\text{H}_{10}\text{O}_5 + 12\text{O} = 6\text{CO}_2 + 5\text{H}_2\text{O}$ .

*Forms of Occurrence*.—Free oxalic acid in solution may be detected by the addition of calcium chloride, when calcium

oxalate will be deposited. Fortunately, when oxalic acid is present in abnormal quantity in the urine, it is always combined with calcium and deposited in the crystalline form. Crystallized calcium oxalate contains water, and is represented by the formula,  $\text{CaC}_2\text{O}_4 + \text{H}_2\text{O}$ . It generally forms in quadratic octahedra with one axis shorter than the other. These crystals are colorless and have sharp angles. Besides this form, calcium oxalate may be found in diamond-shaped crystals, in dumb-bells, or in discs. This salt is insoluble in water, alcohol, ether, alkalis, alkaline carbonates and acetic acid; soluble in hydrochloric, nitric and sulphuric acids, also soluble to some extent in solutions of the acid phosphate and urate of sodium.

Of the four crystalline forms mentioned above, the octahedral is the most common and the most characteristic. Even when other forms are present, some octahedra will generally be found and indicate the nature of the deposit. The only other substance in the urine that crystallizes in octahedra is sodium chloride, and this may always be distinguished from crystals of calcium oxalate by the solubility of sodium chloride in water.

Diamond-shaped crystals may be either uric acid or calcium oxalate, consequently in all cases of doubt, the chemical test should be resorted to; a drop of hydrochloric acid should be added, when the crystals, if calcium oxalate, will be dissolved; if uric acid, they will remain undissolved; or a drop of potassium hydrate may be added, and would dissolve any uric acid, but be without immediate effect upon the calcium oxalate. The dumb-bells may be oxalates, urates or carbonates. A drop of acetic acid would dissolve the carbonates; while the oxalates would be insoluble in acetic acid, but soluble in hydrochloric acid. By the action of either the acetic or hydrochloric acid the urates would be converted into free uric acid which would take a crystalline form and remain undissolved. Discs may be either carbonates or urates, as well as oxalates, and the true nature of such a deposit is to be ascertained by the application of the chemical tests as already given.

*Preparation.*—The beginner should always prepare crystals of calcium oxalate and study them closely before he ventures

to analyze specimens for diagnostic purposes. These crystals may be prepared by adding a few drops of a dilute solution of oxalic acid to some normal urine (200 c. c. or more). After this has been standing for some hours, octohedral crystals of calcium oxalate will be deposited, and may be found on examination of a drop of the urine taken from the bottom of the beaker and placed under a microscope. Care must be taken to avoid adding an excess of oxalic acid to the urine; for if this is done the oxalate is thrown down in an amorphous condition, or the crystals will be imperfect.

*Detection in the Urine.*—Within a greater or less time after emission, normal urine will deposit calcium oxalate; consequently, the examination for this substance in the urine should be made within 48 hours after emission, in order to be of any value for diagnostic purposes. If it be desired to make a close examination of a specimen for calcium oxalate, the 24 hours' urine should be collected, immediately placed in a conical vessel, allowed to stand for 12 hours, and then a drop of the urine from the bottom of this vessel examined under a microscope which magnifies at least 300 diameters, and whose defining power is good. It should be remembered that a natural (one to which nothing has been added after emission) specimen of urine never (with the exception of those passed after the administration of large quantities of oxalic acid) contains sufficient calcium oxalate to form a visible deposit. Urine containing this substance is generally acid in reaction, and the most common accompanying deposits are uric acid and urates.

§ 167. *Physiology.*—Many articles of food contain oxalic acid and other substances which may be converted into oxalic acid during their passage through the body. Oxalic acid taken in the food is partially or wholly oxidized to carbonic acid as it passes through the body. Buchheim and Piotrowsky have shown, by experiments upon themselves, that when from one to seven grams of oxalic acid, as free acid or combined with an alkali forming a soluble oxalate, were taken into the stomach in divided doses within from six to eight hours, from eight to fifteen per cent. of the acid could be recovered from the urine.

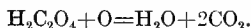
In these cases the urine contained a visible deposit of calcium oxalate, which was in some instances amorphous, in others crystallized in dumb-bells. When calcium oxalate was taken into the stomach in quantity sufficient to contain seven grams of oxalic acid, only from one to two per cent. of the acid could be recovered in the urine. It must be remembered that these quantities were taken in divided doses, and that the administration of three or four grams of oxalic acid in one dose would probably prove fatal.

It is well known that rhubarb contains oxalic acid in considerable quantity; now I found that when five young men, all in apparent good health, ate the same quantity of rhubarb, and the urine of the next twenty-four hours was collected and examined, that the excretion of two of the men contained a crystalline deposit of calcium oxalate; while in the urine of the others, no oxalic acid could be detected. I suppose that in the three, in whose urine no oxalic acid could be found, the oxalic acid of the food was oxidized during its passage through the body. The peculiarities of individuals upon the elimination of this acid unchanged, when taken with the food, or for purposes of experimentation, need further investigation.

It is often desirable, as in the experiments mentioned above, to determine the amount of oxalic acid excreted during twenty-four hours. For this purpose, collect the urine and concentrate it to one-sixth its volume on the water-bath or steam-bath. Render the concentrated fluid strongly acid with acetic acid in order to hold the earthy phosphates in solution, then add calcium chloride which throws down any oxalic acid as calcium oxalate. Allow the precipitate to stand for forty-eight hours, then collect it upon a filter, wash with a little water and then dissolve in hot hydrochloric acid. This solution, filtered, in order to remove any uric acid, is neutralized with ammonium hydrate, then acidified with acetic acid. The calcium oxalate, which is now precipitated, is collected upon a weighed filter, dried at  $120^{\circ}$  and weighed. As a confirmatory result, the calcium oxalate may be redissolved in hot hydrochloric acid and the calcium precipitated from this solution, by

the addition of dilute sulphuric acid and alcohol, as calcium sulphate,  $\text{CaSO}_4$ . This may be collected, dried, ignited and weighed, and from this the amount of calcium and its corresponding amount of oxalic acid may be calculated.

That oxalic acid in the system results from the imperfect oxidation of other substances is now considered as an established fact. It is one of the intermediate stages in the process of retrograde metamorphosis, and may result from either the starchy, fatty, or albuminous food. It is probably present in small quantities in normal blood, but its physiological existence is of short duration, and the matter of which it is composed normally passes on to the production of carbonic acid and water, as represented by the following equation:



§ 168. *Pathology.*—The continued presence of a deposit of calcium oxalate in the urine is indicative of a condition of the system designated by the term *oxaluria*. It must be remembered that an occasional deposit of calcium oxalate may occur in the urine of a healthy person, and it must be repeated here that *all examinations of the urine for the detection of this substance, in order to be of any value in diagnosis, must be made within forty-eight hours after the emission of the urine.* No doubt that many a patient has been treated for oxaluria, when the formation of calcium oxalate in his urine was due to changes going on in the urine after emission, and having no connection whatever with any condition of the patient.

From what has been given concerning the chemistry and physiology of oxalic acid, it will not be difficult to understand some of the circumstances which may lead to oxaluria. In the first place, indigestion is a frequent cause of the appearance of oxalates in the urine. The food is but partially fitted for absorption and the processes of oxidation are retarded just so much. In these cases, the indigestion must be treated. The cause of the indigestion must be sought. It may be that the patient's food is not of the right kind; it may contain so much starch that all of it cannot be completely oxidized by the oxygen of the oxyhæmoglobin; or the patient may be breathing

impure air and the oxygen of the blood may not be sufficient to oxidized a normal amount of food. Again the excessive use of alcohol is a frequent cause of oxaluria; this is true for two reasons, (1) the excessive use of alcohol deranges digestion, (2) the alcohol furnishes the oxygen of the blood a fuel more readily consumed than that furnished by the solid food; consequently, the latter is only half burned and that which should pass off as gas (carbonic acid) through the lungs, falls as cinder (oxalic acid) through the kidneys. The physician must investigate the conditions under which his patient lives. For the purpose of assisting in the oxidation of the food, and stimulating the action of the liver upon the food, I know of nothing better than nitro-muriatic acid. In cases of nervous prostration, this acid may be given with strychnia and other tonics. The nitro-muriatic acid is much more efficient when it is kept undiluted until it is used; then from three to five drops should be added to a tumbler of water, the mixture is then stirred and taken through a glass tube. Thus prepared, it forms a slightly acid, pleasant drink; but very few patients will have anything to do with the strong acid; they observe the color and odor of the chlorine that is given off when the stopper is removed, and then cry out in holy horror against the "terrible thing." In truth this preparation needs to be handled with care, for a drop upon any article of clothing will soon destroy the texture. Consequently, it is better that the physician should prescribe the less efficient *acidum nitro-muriaticum dilutum* of the pharmacopœia to the majority of his patients. This should be given in doses of from 10 to 15 drops in water as recommended for the other form. It must be remembered that the dilute acid should be frequently renewed, as it soon loses its chlorine and oxides of nitrogen upon which its virtue depends. Both the dilute and stronger preparations should be kept in well-stopped bottles and protected from the light.

In phthisis, emphysema of the lungs, and pneumonia there is frequently a deposit of calcium oxalate in the urine. This arises from a deficient supply of oxygen and the oxalates are

frequently accompanied by crystals of free uric acid and deposits of acid urates. In cases of venous stasis arising from disease of the heart or lungs, oxalates, uric acid and urates are deposited. It is well known that this condition of venous stasis causes chronic hyperæmia of the kidneys and that albumen then appears in the urine; but often long before the appearance of the albumen, the urine will contain oxalates, urates and uric acid in deposit.

In skin diseases, oxalates and uric acid are almost invariably present in the urine either as an occasional or constant deposit. The frequent occurrence of uric acid and calcium oxalate in the urine in eczema and psoriasis has led some to believe that, in health, considerable quantities of soluble urates and oxalates are excreted by the skin. They find the amounts of these substances in the urine increased in these diseases, and conclude that this increase is due to the supposed fact that the uric and oxalic acid, which normally pass out through the skin, are now forbidden that avenue of escape and, consequently, are present in the urine. Anything which interferes with the action of the skin, correspondingly retards oxidation and this, no doubt, is the true explanation of the increase of uric acid and calcium oxalate in eczema, etc.: for in all these cases the amount of urea is diminished. Only in suppression of urine, is it positively known that the skin excretes either urea, uric acid, or oxalic acid, and in suppression, any or all of the urinary constituents may be present either in the fæces, perspiration, vomited matters, or pulmonary exhalations.

The continued presence of an excess of oxalic acid in the body is sometimes accompanied by a greater or less disturbance of the nervous system. The patient often becomes very much alarmed and fancies that he will soon die. One day, he will complain of a severe headache and will imagine that his brain is diseased; probably within less than 24 hours, he will again summon his physician requesting that his heart be examined, thinking that *that* organ is diseased; but more frequently the patient's attention is called to the urinary organs. He becomes irritable, dejected and is unreasonable in his

desires and demands. Upon examination of the urine of such a person, a few octahedral crystals of calcium oxalate will generally be detected. Such cases demand the most serious attention of the physician; but I regard these peculiar symptoms as evidence of a diseased condition of the imagination rather than of any serious disorder of the body. These symptoms often occur when no oxalic acid can be detected in the urine; while on the other hand there may be a constant and abundant deposit of calcium oxalate without the appearance of these nervous disturbances. Therefore we cannot regard the presence of an excess of oxalic acid either as the sole cause or constant indication of the symptoms. The treatment must be determined by the peculiarities of the individual case and its discussion belongs to nervous pathology.

In other cases, the first complaint is of want of energy, sick-headache, pain in the region of the kidneys and bladder with frequent desire to micturate. In a typical case of this kind, the urine will be strongly acid and, when passed, will often be cloudy; on standing, quite a deposit forms and will be found to consist of finely divided pieces of epithelium. This deposit is without any form and is generally supposed to be mucus; but chemical examination will show that it contains no mucin. For the microscopical examination of this deposit, a good microscope with a magnifying power of 400 diameters and, what is more essential, with good defining power; is needed; moreover, a trained eye and a skillful hand are quite essential. With these requisites, such a deposit will be found to contain besides the amorphous pieces of epithelium, numerous minute octahedra of calcium oxalate. I have mentioned a skillful hand as one of the requisites in this examination; the importance of this aid will be appreciated when we remember that the detection of these crystals often depends upon the skill with which the fine adjustment of the instrument is moved in order to catch the reflection from the sides of the octahedron. It is true that often large octahedral crystals will be found in these deposits, but I am of the opinion that these result from the growth of the smaller ones after emission; because, if the specimen be



examined within an hour after it has been passed, only the minute crystals are present, while after several hours have elapsed, many large ones will be found.

These minute crystals with their sharp points pierce and irritate the walls of the bladder and the substance of the kidney. That these crystals penetrate the substance of the kidney can hardly be questioned; indeed they have been found in this situation by Crosse and Meckel; while they have been detected in the blood by Garrod. The pain caused by these crystals in the kidneys and bladder is constant and dull; but often so marked as to cause both patient and physician to believe that there is structural disease of the kidney. Several times when physicians have requested me to make examinations of specimens of urine for albumen and casts, saying that they knew their patients to have "Bright's disease" and only wanted to know the proportion of albumen and the nature of the casts, I have found neither albumen nor casts, but a great abundance of these minute crystals. If such a case be taken under care at this stage, relief may be secured with certainty. Plenty of fresh air and good water, especially should this be free from lime, with proper food and nitro-muriatic acid will seldom fail to remove the oxalates from the urine and the pain from the kidneys and bladder. However, there is one word of caution that must be given here. In some of these cases, there is but little urine (from 400 c. c. to 800 c. c.) passed during the 24 hours, and this is strongly acid. Now if the irritation has existed for any length of time, all the nitro-muriatic acid, that can be given the patient, will not bring relief until the quantity of urine is increased. In such cases, the patient should be requested to drink much water, and proper diuretics should be administered.

If the formation and consequent irritation of these oxalates be allowed to continue, one or both of two very serious results may follow. These are (1) structural disease of the kidney, and (2) the formation of a stone. The continued irritation produced by these crystals is not an unfrequent cause of parenchymatous inflammation of the kidney. Year after year the irritation may continue, and finally the substance of the kidney

begins to break down; this organ soon becomes incapable of performing its function, and death results.

Calculi composed exclusively of calcium oxalate are very rare. Many calculi contain this substance as a constituent, and may consist principally of it; but there is generally either uric acid or phosphates, or both, present. Uric acid and calcium oxalate coexist in the same stone so frequently, because both result from deficient oxidation, and may depend upon the same cause. Calculi of calcium oxalate are often coated with a layer of phosphates. The oxalic stone is very rough and presents many protruding points, indeed so marked is its irregular surface that the term *mulberry calculus* has been used to designate a stone composed of calcium oxalate. Now such a stone cannot exist for a long while in any part of the urinary tract without causing considerable irritation. If it be in the bladder, the walls of this organ closing down upon the stone, when the urine is forced through the urethra, are wounded by the rough surface of the calculus; consequently, cystitis often follows, the urine becomes ammoniacal, phosphates are thrown down and deposited upon the stone. If the calculus of calcium oxalate be formed in the pelvis of the kidney, pyelitis and often occlusion of the ureter result; the urine is retained either in part or altogether, and decomposition of urea with consequent deposition of phosphates follows. In this way a small calculus of calcium oxalate may receive layer after layer of phosphates upon its surface and become a large stone.

Suppose that a stone of calcium oxalate has formed, is there any medicinal agent by which it may be removed? In considering this question, we will suppose that the calculus is in the kidney; for if it be in the bladder, it would be very unwise in the physician and unjust to the patient to depend upon the slow and uncertain action of medicines given by the stomach or injected into the bladder, when the knife of the surgeon affords a speedy and certain removal. But cutting down upon, and thus removing a stone from the kidney has been attempted as yet but a few times, and has been attended with but partial success. Consequently, the physician must do the best he can,

and the line of treatment which I have followed with some success is briefly as follows: In the first place, all lifting of heavy weights or any thing which may cause a strain upon the small of the back is positively forbidden. From three to five drops of the strong nitro-muriatic acid are given after each meal as already directed. This is done to prevent the further deposition of calcium oxalate. From one to two hours before each meal, from one to six drachms of sodium phosphate are given in a broth. This is done in order to dissolve the stone already formed. For the phosphate of sodium, the carbonate or citrate of this base or potassium may be substituted. If there be only small pieces of gravel of calcium oxalate in the kidney, this treatment long continued will be found beneficial; but if there be a large stone, one of a quarter of an inch or more in diameter, I know of no medicinal agent which will remove it.

Hassall and Beale think that the dumb bell form of calcium oxalate forms renal calculi more frequently than the octahedral variety. I have found the dumb-bell form constantly in the urine in two cases of renal calculi; but have observed the octahedral constantly present in a large number of cases; while in one instance, sometimes one form and again the other, and at still other times, both would be present in the deposit.

#### XANTHIN,— $C_5H_4N_4O_2$ .

§ 169. *Properties*.—Pure xanthin is a glistening, white, amorphous powder, which becomes wax-like on being rubbed. It is practically insoluble in water, one part of xanthin requiring as much as 14000 parts of cold, and 1400 parts of hot water for solution. It is also insoluble in alcohol and ether, but soluble in the caustic alkalis and the mineral acids. It has feeble basic properties, and forms salts with the strong acids. If a solution of xanthin be evaporated on the water-bath, the xanthin is deposited in crystalline scales. From a concentrated ammoniacal solution, xanthin is precipitated on the addition of silver nitrate as  $Ag_2OC_5H_4N_4O_2$ . This precipitate is soluble in hot nitric acid, from which is deposited on cooling xanthin-silver nitrate,  $C_5H_4N_4O_2AgNO_3$ . The ammo-

niacal solution of xanthin is also precipitated by lead acetate, calcium chloride and zinc chloride.

*Tests.*—(1) If a watch-crystal be partially filled with a solution of sodium hydrate, some calcium hypochlorite be added, and the mixture be well stirred, then a little xanthin be added, a dark-green ring soon forms around the spot where the xanthin was dropped; this color soon changes to a brown and finally disappears.

(2) If some xanthin be placed in a clean porcelain dish, covered with a few drops of nitric acid, and then heated to dryness, a yellow residue remains. If this residue, while yet warm, be treated with a drop of sodium or potassium hydrate solution, a deep purple color is developed; but the purple is not produced by ammonia, (means of distinguishing from uric acid).

(3) In dilute solutions of xanthin phosphomolybdic acid produces an abundant yellow precipitate. This precipitate is soluble in hot dilute nitric acid from which it separates in cubes on cooling.

*Preparation.*—(1) Stædeler recommends the following method of obtaining xanthin from muscular tissue, or from the heart, liver, or spleen: Cut the organ or tissue into fine pieces; rub these up in a mortar with ground glass; add dilute alcohol to the pulp, stir, warm, and press through cloth; digest the residue for an hour with water at 50° and again filter through cloth; unite the alcoholic and aqueous extracts and remove the alcohol by distillation. Filter the remaining fluid in order to free it from coagulated albumen; concentrate the filtrate and add to it, first some lead acetate, then basic acetate of lead, and after it has stood for some hours, add mercuric oxide. Suspend the precipitate formed by the mercury and lead, and treat with a current of hydrosulphuric acid gas; remove the precipitated sulphides by filtration, and evaporate the filtrate to dryness on the water-bath, when xanthin and hypoxanthin remain. If this residue be treated with cold, dilute hydrochloric acid, the hypoxanthin will be dissolved, and may be removed; while the xanthin remains insoluble.

(2) Xanthin may be obtained from normal urine, but it is

present in quantities so small that large quantities of urine must be used in its preparation. The method proposed by Neubauer for obtaining xanthin from normal urine is as follows: Concentrate from 100 to 200 pounds of urine on the water-bath; treat with the baryta mixture and filter in order to remove phosphates and sulphates. Concentrate the filtrate to a syrup and allow to stand for some time: decant the supernatant fluid from the salts which have separated by crystallization; dilute this fluid with a little water and add copper acetate; boil this mixture for a short time, and collect the dirty brown precipitate, which has formed, on a filter; wash with cold water until the wash-water no longer contains chlorine (tested for with silver nitrate); dissolve the precipitate with warm nitric acid and add to this solution some silver nitrate, which reprecipitates the xanthin; dissolve this precipitate in hot, dilute nitric acid and filter while hot. As the filtrate cools, xanthin-silver nitrate will be deposited. This compound, freed from nitric acid by being digested with ammonium hydrate, is treated with hydrosulphuric acid, and filtered while hot. The filtrate on cooling deposits impure xanthin, which may be purified by solution in hot nitric acid, and filtration through animal charcoal. This filtrate is neutralized with ammonium hydrate, evaporated to dryness and the residue is washed with water, which removes the ammonium salt, while the xanthin remains pure and insoluble.

§ 170. *Physiology*.—Xanthin is found in the various tissues of the body, having been obtained from the liver, pancreas, spleen, muscles, and blood. It is an intermediate product of oxidation; although it has never, in the test tube, been directly oxidized to uric acid. Rheineck has reduced uric acid to xanthin with a very dilute solution of sodium amalgam; while on the other hand, xanthin is obtained by the action of nitrous acid on either guanin or hypoxanthin. Xanthin, in very small quantities, is a constituent of normal urine, the daily amount not being more than one grain. It exists in large proportion in the excrement of spiders.

§ 171. *Pathology*.—In two instances, I have found xanthin

deposited with uric acid in the urine of patients with enlarged spleen. In one of these cases, the daily excretion of uric acid was as much as 23.5 grains. The deposit, which was quite heavy, consisted of urates, uric acid and xanthin; these were separated by dissolving in strong sulphuric acid, and then diluting with water; when the uric acid was reprecipitated, and the xanthin remained in solution. Although xanthin, as prepared from muscle and normal urine, is granular and amorphous, when in great excess in the urine, it is deposited in small oval crystals. Prof. Langenbeck once extracted a calculus, the size of a hen's egg, which on analysis was found to consist entirely of xanthin. Whether pieces of gravel contain xanthin or not may be ascertained by the tests, (1) with nitric acid and potassium or sodium hydrates; (2) with sodium hydrate and calcium hypochlorite, and (3) by their ready solubility in ammonium hydrate.

The only known injurious result of an excess of xanthin in the urine is the formation of stone.

#### HYPOXANTHIN, $\text{—C}_5\text{H}_4\text{N}_4\text{O}$ .

Hypoxanthin, known also as sarkin, has been found as a normal constituent of muscles, and of the substance of the liver, spleen, lungs, and marrow of the bones. In the blood and urine of leucocythæmia, hypoxanthin is present in abnormal quantity. It resembles xanthin very much in its reactions and is a true animal alkaloid, uniting with acids to form salts. Hypoxanthin forms in fine, microscopical needles, which are soluble in 300 parts of cold, and 78 parts of hot water; insoluble in alcohol. It is freely soluble in the caustic alkalis and the mineral acids.

If an ammoniacal solution of hypoxanthin be treated with silver nitrate, a double salt of silver and hypoxanthin is precipitated. This salt has the formula,  $\text{Ag}_2\text{OC}_5\text{H}_4\text{N}_4\text{O}$ , and forms a gelatinous mass. If an aqueous solution of hypoxanthin be treated with silver nitrate, a precipitate having the composition represented by the formula,  $\text{C}_5\text{H}_4\text{N}_4\text{O Ag NO}_3$ , is thrown down, and will be found to be soluble in hot, strong nitric acid, from which it falls in crystalline scales on cooling. Hypoxanthin

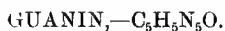
forms double salts with some other bases, among which are barium, copper, and platinum.

Of the mineral acids, hydrochloric is the best solvent for hypoxanthin. This solution consists of the formation of the chloride of hypoxanthin, and if it be evaporated to dryness on the water-bath, this salt remains in glistening tablets. From its solution in the alkalis, hypoxanthin is precipitated by a current of carbonic acid gas. By the action of oxidizing agents as nitrous acids, hypoxanthin takes another atom of oxygen and is converted into xanthin. The basic properties of this substance are quite marked, and its chloride, nitrate, sulphate, and other salts have been closely studied by Thudichum and others.

§ 172. *Physiology*.—Hypoxanthin is formed by the oxidation of guanin, and we have here a physiological chain, the known links of which are guanin, hypoxanthin, xanthin, uric acid and urea. These are stages through which nitrogenous constituents of our food and tissues pass on their return to the inorganic world. Each of these contains  $C, H, N$ , and  $O$ ; but there is a progressive increase in the oxygen until urea is reached, and then one step further carries this once highly organized matter back to inorganic nature. Under the influence of the heat of fever, the urea is sometimes converted into ammonium carbonate within the body. Thus, the tissues of the fever patient may really be burned to ashes. I look forward to the time when the physiologist will be able to trace matter from the inorganic world, through all its various changes in the plant and animal, until it returns to dust. If such knowledge be ever attained, the physician will endeavor to ascertain two things: (1) the means of preventing arrest in these progressive changes, and (2) the means of preventing the too rapid transformation of matter. Many diseases arise from each of these causes: thus in cholera, there is arrested transformation. Life depends upon the liberation of force resulting from the oxidation of the food. Stop this oxidation, or process of force liberation, and life for that individual ceases. But in the majority of cases, the processes of life are not suddenly arrested; but are

retarded and gradually brought to a stop. The fire is not immediately extinguished, but the cinders and ashes are allowed to accumulate and shut out the air. On the other hand, in all acute febrile diseases, the transformations go on too rapidly; too much force is liberated, and the tissues of the body are consumed in this over production of force. Says Prof. Haughton, "An additional amount of work, equivalent to the body lifted through nearly one mile per day, is spent in maintaining its temperature at fever heat. If you could place your fever patient at the bottom of a mine, twice the depth of the deepest mine in the Duchy of Cornwall, and compel the wretched sufferer to climb its ladders into open air, you would subject him to less torture, from muscular exertion, than that which he undergoes at the hand of nature, as he lies before you, helpless, tossing and delirious, on his fever couch."

§ 173. *Pathology*.—Hypoxanthin has been found deposited in the urine in severe diseased conditions of the liver, spleen, and kidney.



§ 174. It is not positively known that this substance ever occurs in the urine; but it is of value here on account of its relation to xanthin, hypoxanthin, and uric acid. Guanin is present in Peruvian guano, from which it may be easily obtained. It has been found combined with calcium in the scales of some fish, and has also been extracted from the muscle, liver, and pancreas of man. It has been detected in the muscles, tendons and joints of diseased pigs.

*Preparation*.—Boil Peruvian guano with water and milk of lime until some of the filtered solution is colorless; then filter through cloth. Urea and some other substances are contained in the solution; while uric acid and guanin remain undissolved. Now boil the residue with a solution of sodium carbonate repeatedly, until the filtered fluid ceases to give a precipitate on the addition of acetic acid. The united filtered extracts, made with the solution of sodium carbonate, are treated with acetic acid, until a decidedly acid reaction is obtained. This precipitates the uric acid and guanin. The precipitate is



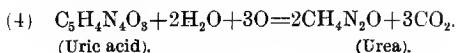
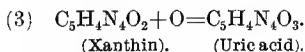
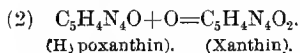
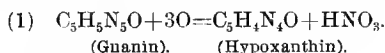
allowed to stand for 24 hours, then the supernatant fluid is removed either by decantation or filtration. The residue is boiled with hydrochloric acid and filtered. The guanin being soluble in hydrochloric acid, passes through the filter, while the uric acid remains insoluble. From its solution in hydrochloric acid, the guanin is precipitated on the addition of ammonium hydrate.

*Properties.*—Guanin is a white, amorphous, odorless, tasteless powder, which is insoluble in water, alcohol, ether and ammonium hydrate. It is soluble in the mineral acids and in sodium and potassium hydrates. With the mineral acids, guanin forms crystalline salts, the best known of which is the chloride; this salt also forms double salts with several bases, among which are mercury, platinum, and zinc.

*Tests.*—If some guanin be placed on platinum foil, a few drops of nitrous acid be added and then heated to dryness, a yellow residue remains and by caustic soda is colored red: this color being changed to a purple on the application of heat. By means of nitrous acid, guanin is converted into xanthin; while by potassium chlorate and hydrochloric acid, it is converted into xanthin, parabanic acid, and guanidin,  $\text{CH}_5\text{N}_3$ .

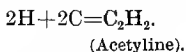
§ 175. *Physiology.*—If guanin be taken into the stomach, it is oxidized as it passes through the body, and increases the amount of urea. But there is a limit to this oxidation of guanin in the body, and if very large quantities be taken, all of it is not excreted as urea.

The relations between the different compounds, which have been studied here, and which arise during the retrograde metamorphosis of nitrogenous food and tissue, are best represented by the following equations:

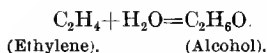
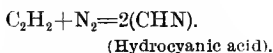
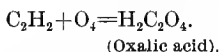
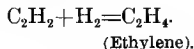




passed between carbon poles in an atmosphere of hydrogen, when these two elements unite and form acetylene:



From acetylene, a variety of organic substances may be built up, as represented by the following equations, taken from the Lehrbuch of Gorup-Besanez:



Chemical force, or chemism, is that which causes atoms to unite or to rearrange themselves to form molecules; and every molecule, simple or compound, inorganic or organic, formed in air, water, earth, plant, or animal, is produced by the *chemical* combination of its atoms. Every change in the atomic arrangement of any substance during absorption, assimilation, or excretion is due to chemical force. Every proximate principle formed in the plant or in the animal is a chemical formation.

Although the formation of every molecule is due to chemism and it is the province of chemistry to study these formations, the building up of these molecules into cells is an entirely different thing and does not fall within the domain of chemistry. Why certain molecules form liver cells, while others produce bone cells, and still others, nerve cells, is a subject which in no way concerns the chemist. It is a chemical fact that oxalic acid precipitates calcium from its solution in normal urine, but why the molecules of calcium oxalate unite so as sometimes to produce octahedral, and at other, dumbell, at still other, diamond-shaped crystals is no part of chemistry to investigate. The formation of the crystal is to be studied in the laboratory of physics.

#### ALBUMEN.

§ 176. Urine containing albumen may be either of high or

low specific gravity, though, generally, if much albumen be present, the specific gravity will be low. If sugar be present with the albumen, the specific gravity may be high; again, in certain structural diseases of the kidneys and even in the later stages of these diseases, the urine may be very dense on account of the small amount of water present. In amyloid degeneration of the kidney, I have seen the specific gravity of the 24 hours' urine rise to 1040 just before death. In this case, the total urine for the 24 hours did not measure 200 c. c. From this, we see that a high or normal density is not proof sufficient of the absence of albumen.

*Heat and Nitric Acid Test.*—The best test for this substance is heat and nitric acid; first applied separately and then combined. Heat coagulates albumen; but if we rely on this test alone, we will sometimes overlook it when present, and at other times, get a cloudiness when the solution contains no albumen. If the solution be neutral or alkaline, heat will often fail to coagulate the albumen until an acid has been added. In many cases, on heating a specimen of urine, a cloudiness, due to the precipitation of phosphates, appears; but redissolves on the addition of a drop of nitric acid. On the other hand, nitric acid alone may in some instances throw down a precipitate of either nitrate of urea or acid urates, which would be redissolved on the application of heat. Consequently, the best way is to apply heat to one part, nitric acid to a second, and both heat and nitric acid to a third. Another chance of error here lies in the fact that a coagulum of albumen may be redissolved on the addition of nitric acid, if either too little or too much of the acid be added. If but little nitric acid be added and there be an excess of phosphates present, the nitric acid unites with the bases and forms free phosphoric acid; now albumen is soluble in free phosphoric acid, and more nitric acid must be added to reprecipitate the albumen from this solution. An excess of nitric acid added to a faint cloud of albumen may form an acid-albumen which passes into solution. In order to avoid these sources of error, it is well to add the nitric acid slowly, a drop at a time; and to about a drachm of urine, in a

test tube, from three to fifteen drops of the ordinary reagent, nitric acid, should be added.

The method of procedure in applying this test when only a trace of albumen is present is of the greatest importance, and is best as follows:

(1) Apply heat to the upper portion of some urine in a test tube and gradually raise the temperature to the boiling point. The coagulation of the albumen by the heat renders the fluid turbid, which is more easily recognized by contrast with the clear portion beneath. If the urine be cloudy with urates, these will be dissolved by the heat before the albumen is coagulated. The heat test is sufficient when the urine is cloudy with urates; but in all other cases it should be supplemented by the nitric acid test. (2) Incline a test tube containing about an inch in depth of the urine and allow the nitric acid to flow down the side of the tube to the bottom. If albumen be present the urine will become turbid just above the layer of acid; while if much albumen be present the fluid will be rendered turbid throughout. If but a trace of albumen be present, the opalescence may not appear for some minutes, and will only form a thin layer just above the acid, while the upper portion of the urine remains unaltered. (3) The turbidity produced by nitric acid should not disappear on the application of heat.

It is necessary to remember that albumen, when present in the urine, is in solution and never in deposit. Albuminous substances, as mucus, may be deposited, but true albumen is in solution, and the test must always be applied to the clear fluid. If any deposit be present, it must be removed either by filtration or decantation. Often the urine will be cloudy from suspended mucus which will not fall as a deposit; in such cases the urine must be filtered, and if necessary, must be passed through several filter papers until it is clear. After a perfectly clear fluid has been obtained, apply the test with heat and nitric acid, and then if any coagulum or cloudiness appears and remains (when tested as recommended, with both heat and nitric acid), the presence of albumen is certain. Even in structural diseases of the kidneys, as renal cirrhosis, the amount of

albumen is often very small, and only sufficient to produce a distinct opalescence, when the urine is treated with heat and nitric acid.

§ 177. *Physiology*.—When we speak of albumen in the urine, we mean that kind which is precipitated by heat and nitric acid; for there is a variety of albumen, precipitated by chloroform and absolute alcohol, which is a normal constituent of the urine. We must constantly bear in mind that there are many kinds of albumen, and if certain of these get into the circulation, they are unfit for use in the body and must be excreted; this accounts for the temporary albuminuria caused by indigestion. Sometimes after one has eaten a large meal, especially if it consisted of food not easily digested, and be taken late in the day, albumen temporarily appears in the urine. Also if excessive exercise be taken soon after a meal, or indeed at any time of the day, albumen may be present in the urine even in considerable quantities. Moreover, in cases of this kind, hyaline and epithelial casts have been known to appear in the urinary deposits. Albumen in the urine may be due to the presence of blood or pus, as in hæmituria and cystitis, or directly from the serum of the blood as in structural diseases of the kidneys.

#### PATHOLOGY.

##### (a) HÆMATURIA.

§ 178. Blood in the urine may be detected by the color, by microscopical examination, or by the spectroscope. Bloody urine is always albuminous. The color will vary from bright red to a smoky or even a black tint. The fluid is dichroistic, red by transmitted and green by reflected light, if much blood be present. The corpuscles may generally be detected by the microscope; but sometimes they are completely disintegrated, then the spectroscope may be used to advantage. The source of the blood can almost invariably be ascertained. If there be clots large enough to be visible to the unaided eye, the blood must have passed into the urine below the secreting structures. If from the bladder, the clots will often be quite large and may obstruct the passage through the urethra. If the coagula-

tion has taken place in the ureter, the shape and size of the clots will so indicate. These have been mistaken for entozoa. When from the pelvis of the kidney, the coagula are much smaller than those from the bladder, and may preserve the shape of the calices. If from the substance of the kidney only, the clots will be microscopic in size, having been formed in the tubules, and the urine will generally have a smoky tint. Profuse bleeding from a wounded kidney or a highly vascular cancer of that organ may cause large clots to be formed in the pelvis of the kidney, or in the bladder. When the urine has a bloody color and no corpuscles can be found, and it is not convenient to apply the spectroscope, Heller's test for blood-pigment may be used. This consists in boiling the urine with a solution of sodium hydrate, when the earthy phosphates will be precipitated and will entangle the blood-pigment forming a brick-dust or red deposit. But the spectroscopic examination is by far the best method of testing for blood-coloring matters.

(b) PUS AND EPITHELIUM.

§ 179. Whenever there is sufficient pus in the urine to give a compact deposit, it could not have come from the kidney only, since in the severest inflammation of the kidney substance the amount of pus formed is small. If the pus be from the bladder, it will contain more mucus, be ropy, and the urine will generally be alkaline from a volatile alkali; the urea having been decomposed, while in the bladder, into ammonium carbonate. This decomposition takes place very rapidly, as the mucus, which is poured from the irritated walls of the bladder, acts as a ferment. In suppurative cystitis, the greater part of the pus and mucus is passed after the water, while in pyelitis the pus will be distributed through the urine, which will generally be of acid reaction. There is a chance of making a very serious mistake here: suppose that a specimen of urine be found to be acid and to contain traces of pus. Now this pus may have come from the kidney or from the urethra, and often it will be impossible to decide from an analysis of a specimen, collected in the usual way, whether there be a diseased condition of the kidney or simply an inflamed condition of the urethra. This

question can be decided with certainty only by adopting the following procedure: when the patient goes to urinate, the first drachm or two passed should be collected in a small clean beaker or other vessel, and the remainder of the urine in a second vessel. Now if the pus be from the urethra, the urine first passed will wash it all out; while the second portion of the urine will contain no, or very little pus. On the other hand, if the pus be from the kidney, it will be distributed in the bladder and the second portion of the urine will contain as much as that passed first. To Sir Henry Thompson, I believe, belongs the credit of first calling attention to this serious source of error in the analysis of urine.

In all these cases, the epithelium should be closely studied. This may be from the uriniferous tubules, pelvis, ureter, bladder, urethra, or vagina. If the individual epithelial cells are normal in appearance, but unduly increased in amount, there is indicated only an excessive desquamation which may be due to simple hyperæmia and may be temporary or even physiological in its nature; thus, during pregnancy, the urine often contains a visible, dirty white deposit, which consists principally of vaginal epithelium. However, if the cells contain oil or are broken, there is some degeneration of the part from which they came. In fatty degeneration of the kidney, the renal epithelium will contain and may consist principally of oil globules. This condition follows poisoning by phosphorus as well as it results from a general diseased state of the system. In these cases, care is needed to distinguish between oil globules in the epithelium and those that may be floating freely through the fluid; the latter most frequently are due to some accidental cause, having been introduced into the urine after its emission. The student should make it a rule to study epithelium closely and decide upon its source in every instance, because these cells are overlooked by some, and mistaken for casts by others.

#### (c) CASTS.

§ 180. It was formerly supposed that all casts were formed by the coagulation of albumen, from the blood-serum, in the uriniferous tubules. But it has been ascertained by chemical



analysis that the different kinds of casts vary in their composition and that the majority of them are not composed of fibrin nor of any protein substance. As they vary in their origin, they likewise vary in their pathological importance. In examining for casts, the greatest care and patience is demanded. It is true that in many instances, they will be found in abundance on every slide examined; but in other cases, it will be necessary to pour the urine into a conical glass and leave undisturbed for from one to twelve hours and then examine the sediment most thoroughly. Having found the casts, it will remain to determine their exact nature and pathological significance. Casts vary in diameter from .01 to .05 of a millimeter, and in the length with the place of formation.

*Epithelial casts.*—These are cylinders or pipes formed by the removal of the epithelia of the tubes in mass. The distinct cells can be recognized, and these casts are caused by inflammation of the mucous membrane.

They may be produced by highly concentrated, acid urine containing urates or other irritating substances.

*Granular casts* consist of a mass of aborted epithelia, differing from the epithelial casts in the fact that the individual cells are not fully developed. In some cases, these granules are closely adherent; while in others, they seem to be on the point of breaking up. The cells are generally as large as a pus cell and somewhat darker.

*Bloody casts* consist of coagulated fibrin with blood corpuscles entangled and are formed in hæmaturia. Under the microscope these appear as dark granular masses.

*Hyaline casts* are smooth, structureless, and consequently often escape detection. They may be detected upon the addition of iodine in potassium iodide, or of a dilute solution of carmine, when they will be stained yellow, or red, respectively. They are narrow, glass-like in appearance, sometimes containing a few fine granules or oil globules, and are formed by the coagulation of albumen in the uriniferous tubules and simply indicate albuminuria. These casts are often found in urine of persons suffering with severe febrile disease, when the post mor-

tem reveals no pathological condition of the kidney whatever.

*Waxy casts* have the appearance presented by melting a piece of wax, dropping it on a glass-slide and allowing it to cool. They are distinguished by their glistening appearance and by being of a faint yellow color; they are formed by an abnormal secretion from the mucous membrane of the kidney. Not unfrequently the largest casts found are of this class.

(d) THE URINE IN DIFFUSE DISEASES OF THE KIDNEY.

(1) HYPERÆMIA.

§ 181. (1) *From Irritant Poisons*.—The poisons, which most commonly produce excessive congestion of the kidney, are cantharides, turpentine, cubebs, copaiba, potassium nitrate, and cardol (oleaginous liquid from the *anacardium occidentale*). All slow poisons, all the effete products of the body, as calcium oxalate which soon disease the kidney, and all those which act directly upon the tissues of the kidney, as arsenic, phosphorus and the mineral acids, are excluded from this list; because they produce other changes than that of simple hyperæmia.

The symptoms are distinctly marked, since in the majority of instances, the irritation is not confined to the urinary tract, but the digestive suffers as well; so that nausea and vomiting will frequently attract attention before the patient's notice has been called to the urinary tract. Here the first manifestation is a desire to pass water at short intervals of time; but the amount of water is not usually increased, and in some instances is notably decreased. Soon the urine becomes bloody, with a greater or less quantity of albumen, and there may be bloody casts with excess of epithelium from the uriniferous tubules. If turpentine is the cause, the urine will have the characteristic odor of violets, and the turpentine can be recognized by its odor in the breath. All this may be caused by the *inhalation* of turpentine; if due to this substance, the symptoms will abate on its removal. If the irritation has been caused by cantharides, so much fibrin will be poured out, in consequence of the irritation of both kidney and bladder, that often clots, large enough

to interfere with micturition, form in the bladder. When due to this poison, the symptoms *do not* disappear upon the removal of the exciting cause; but the urine may continue to be bloody and contain bloody casts for weeks.

(2) *Hyperæmia caused by diseased conditions of other organs.*—The urine will be albuminous; the albumen being forced from the inter-tubular capillaries into the uriniferous tubules by the venous congestion. With the albumen more or less blood corpuscles often pass through. There will be a heavy deposit of urates because the amount of water is too small to hold them in solution, and because the absolute quantity of urates is increased by imperfect oxidation. The urea will be in excess in proportion to the water, but deficient absolutely. From the above-mentioned facts it will be seen that the specific gravity is necessarily high, usually ranging from 1030 to 1035.

## (2) PARENCHYMATOUS INFLAMMATION OF THE KIDNEY.

§ 182. In the first stages of chronic parenchymatous inflammation, the urine may be normal in amount and may contain a normal proportion of urea, the only indication of a diseased state of the kidney being the presence of albumen with the occasional or constant appearance of the narrow or hyaline casts, and often of blood and bloody casts. As the disease progresses, the amount of water increases on the average, but often fluctuates from day to day; thus, one day the quantity of urine may be normal, or even less than the average amount passed by a healthy person of the age, size and sex of the patient; while the next day a great excess of urine may be passed. The specific gravity and per cent. of urea vary inversely with the quantity of urinary water. As the disease progresses, granular casts begin to appear and increase in number day by day. If the disease is not arrested, the granular casts soon outnumber those of the hyaline variety; while the latter may entirely disappear. As the disease progresses, the granular casts become darker in appearance and wider, while waxy casts begin to appear. The waxy casts then increase in number and become wider and often are quite yellow and glisten like wax.

Besides the granular casts there will often be observed in the deposit a large quantity of what are known as granule cells. These are aborted epithelial cells from the tubules of the kidney and may be washed out, as such, or they may result from the breaking up of the granular casts into their constituent granules. Moreover, these granules may contain oil, or may consist principally of globules of oil. The globules of oil will also be observed in the casts not unfrequently. Albumen is always present in chronic parenchymatous inflammation of the kidney, and may be found in quantities as large as five per cent.

(3) RENAL CIRRHOSIS.

§ 183. The daily excretion of urine is generally increased; this, however, is not, by any means, always true. Only frequent, I might say constant, examinations of the urine will enable us to detect this condition of the kidney; because, for days and weeks together, the urine may be perfectly normal. Albumen may or may not be found; though it is generally present, but in a small quantity, during some stage of the disease. If any casts are found, which, so far as my experience goes, is rather the exception than the rule, they are of the hyaline variety. The most constant factor in the analysis of the urine is the small per cent. of urea, with an increase in the amount of water.

In a typical case of renal cirrhosis the amount of albumen is very small, only sufficient to produce a slight opalescence when the urine is tested with heat and nitric acid. The amount of urine for the twenty-four hours generally measures from 2000 c. c. to 3000 c. c. The urine may be almost colorless, is generally feebly acid in reaction, and seldom contains a heavy deposit. In such a specimen the greatest care is needed in order to detect any casts that may be present. It is best to pour from thirty to forty ounces of the urine into a conical glass and allow to stand for several hours and then examine a drop of the urine taken from the bottom of this vessel. Although the amount of albumen in this disease is generally very small, I have known it to be present in as great a quantity as two per cent.

## (4) AMYLOID DEGENERATION.

§ 184. The term amyloid was given to this condition of the kidney for this reason: if a preparation of the diseased organ be moistened with a solution of iodine and examined under the microscope, it will be found to be stained a reddish-brown, which is farther changed to violet on the addition of a drop of dilute sulphuric acid. This test, which resembles so closely the well-known reactions of starch, caused Virchow to believe that it was due to a deposit of some amylaceous substance; but on a chemical analysis, it was found to contain nitrogen, and to belong to the albuminates. This amyloid deposit differs, however, from other albuminous substances by being insoluble in gastric juice.

The amyloid deposit may be removed from an organ by the following process: First, an organ which contains much of the amyloid substance (as shown by the readiness and extent to which it colors, when microscopical sections are tested with iodine and with iodine and sulphuric acid) is needed. Remove, as completely as possible, the blood-vessels and also the gall-bladder, if the liver be under examination. Cut the organ into fine pieces; wash these with cold water, removing the water either by filtration or decantation; boil the residue with water, in order to loosen the connective tissue; treat the residue first with alcohol and then with ether in order to remove fat and cholesterin. Boil the residue, which consists of amyloid substance and cell membranes, with alcohol acidified with hydrochloric acid. This forms a gelatinous mass of the other albuminous substances, but is without effect upon the amyloid. Now digest the yet insoluble residue with gastric juice for some hours at 40°. The other albuminous substances are digested and may be removed from the amyloid, which remains insoluble.

Amyloid is soluble in concentrated hydrochloric acid, forming an acid-albumin, or syntonin. From its solution in strong hydrochloric acid, the syntonin is precipitated on the addition of water. In the caustic alkalis, amyloid dissolves, forming albuminates.

During the first stages of this disease, the urine is increased

in quantity, is clear and contains much albumen. Microscopical examination reveals hyaline casts and epithelial cells from the uriniferous tubules; the epithelium will give the reaction with iodine and sulphuric acid, but the casts are only stained yellow.

As the disease progresses, the amount of urine is decreased, until often the daily quantity does not measure 100 c. c.; the specific gravity goes up as high as 1035 to 1040; the per cent. of urea is increased to four or five, while the daily excretion of urea does not vary much from the physiological standard. The urine becomes dark colored and contains a visible deposit, which on microscopical examination is found to consist of hyaline, granular and large waxy casts.

#### (e) CHYLOUS AND LYMPHOUS URINES.

§ 185. Chyluria, known also as galacturia, is a disorder chiefly confined to tropical countries, and the majority of cases met with in other regions are of persons who have resided at some time in the tropics. A few cases, in which the patients had never visited warmer latitudes, have been reported in England and Germany.

The urine is white, having the appearance of milk; the depth of color varying with the amount of fat from that of skimmed milk to that of rich cream. Sometimes the presence of blood imparts a rose tint. On standing the urine is transformed into a coagulated mass, which later dissolves or breaks into flakes spontaneously. If the urine be agitated with ether the milky appearance is destroyed by the removal of the fat. The ethereal extract on evaporation leaves a residue of fat. Under the microscope chylous urine presents a great number of small granular corpuscles which seem to be identical with those of the chyle. Blood corpuscles are often present. The urine is coagulable by heat and nitric acid, showing the presence of albumen. It is distinguished from a mixture of milk and urine by the absence of casein. The constituents of normal urine are present in proper proportion.

The passage of chylous urine is generally irregularly intermittent. The patient is frequently anæmic and emaciated; but

persons have been known to pass chylous urine for a length of time (in one case 50 years) without any serious derangement of health. In some rare instances coagulation has taken place in the bladder causing great pain.

It will be seen that the principal abnormal constituents present in chylous urine are fat, fibrin factors and albumen. If only the last two are present, the urine is *lymphous* and not chylous. Lymphous urine on standing forms a clear, jelly-like coagulum.

#### COLORING MATTERS.

§ 186. The variations in the color of the urine have already been referred to, and it only remains to briefly describe some of the most important coloring matters which appear in the urine either in health or in disease. To separate the various coloring principles of the urine is a difficult task, and it is probable that some of them are more or less modified during the processes of separation.

*Indigogen*.—This substance, also known as uroxanthin and indican, is found as a normal constituent of the urine of mammals, being especially abundant in the urine of the horse. Dilute acids (slowly in the cold, more rapidly when warmed) decompose indigogen with the formation of indigo-blue and indigo-glucin. The former is deposited from the solution in blue granules, or forms a bluish pellicle upon the surface of the fluid. The indigo-glucin is a syrupy fluid which reduces copper, but is incapable of the alcoholic fermentation. Jaffe gives the following methods of detecting and estimating the indigogen in the urine:

(1) *In urine rich in indigogen, as the urine of the horse*.—To 10 c. c. of the urine, add an equal volume of strong hydrochloric acid, then to this mixture add, drop by drop, a saturated solution of chlorinated lime, when the color becomes successively red, violet, green, and blue. Care must be used in the addition of the solution of chlorinated lime; for if too little of this reagent be added, the blue color will not be produced, while if too much be added, the indigo-blue will be oxidized. The exact quantity of the solution of chlorinated lime necessary to pro-

duce the blue coloration should be ascertained by repeated experiments. If it be desired to estimate the quantity of indigogen, from 200 c. c. to 300 c. c. of the urine is treated with an equal volume of strong hydrochloric acid, and the requisite amount of the saturated solution of chlorinated lime. The mixture is allowed to stand for 24 hours, and then the residue is collected upon a filter, which has been washed with hydrochloric acid, dried and weighed. The residue on the filter is washed with hot water which dissolves the hippuric and benzoic acids, while the indigo remains insoluble. The indigo is now washed, first with dilute ammonium hydrate, then with water, after which the filter with its contained indigo is dried at from 100° to 110°, and weighed. The weight of the filter and contents, less the weight of the filter, will be the weight of the indigo obtainable from the indigogen of the urine taken.

(2) *Urine which is poor in indigogen.*—To 1500 c. c. of human urine, add sufficient milk of lime to produce an alkaline reaction, then add calcium chloride which throws down the phosphates and sulphates. After standing for from 12 to 24 hours, the supernatant fluid is removed by filtration. The filtrate is concentrated (at first on the sand-bath, and then on the water-bath) to a syrup. During the process of evaporation, the reaction of the urine should be tested from time to time, and its alkalinity retained, by the addition of sodium hydrate, if necessary. The syrupy residue is now treated with 500 c. c. of strong alcohol and the mixture is warmed for a few minutes; then poured into a clean beaker and allowed to stand for 24 hours. From the solution, the alcohol is now removed by distillation; the residue is dissolved in water and treated with a very dilute solution of ferric chloride as long as a precipitate forms, but avoiding an excess of the precipitant. The precipitate is removed by filtration; the filtrate is treated with ammonium hydrate which precipitates the excess of iron; the mixture is boiled and the precipitated oxide of iron is removed by filtration. The filtrate is concentrated to 200 c. c., filtered, if necessary, and the indigo is precipitated, freed from impurities, dried and weighed as given in (1).



According to Jaffe, one liter of the urine of the horse yields an average of .152 of a gram of indigo; while a liter of human urine yields only .0066 of a gram.

Besides the test given for indigogen by Jaffe, there are several others for the detection of this substance. One given by Stokvis is as follows: Warm some urine with two parts of commercial nitric acid at from  $60^{\circ}$  to  $70^{\circ}$ , then shake the mixture with chloroform. If indigogen be present in considerable quantity, the chloroform will be immediately colored blue; while spectroscopic examination of the chloroform solution will reveal the absorption band of indigo-blue between C and D.

To from 4 c. c. to 6. c. c. of strong hydrochloric acid in a test-tube, add from twenty to forty drops of the urine under examination and gently heat the mixture, when, if indigogen be present, a violet or blue color will be developed. (Heller's test).

*Indigo-blue*,  $C_{16}H_{10}N_2O_2$ .—This substance is not found in normal urine but results from the decomposition of indigogen. It is not unfrequently observed deposited in blue granules in urine, to which nitric or hydrochloric acid has been added in order to precipitate the uric acid. It may also result from indigogen during the spontaneous decomposition of urine. It generally appears in granules, though sometimes in small plates, and at other times in fine needles.

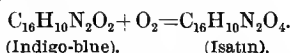
The preparation of indigo-blue from the urine has been given; it may also be obtained from the indigo of commerce. Put some powdered indigo and grape sugar into a clean flask or bottle, add some concentrated solution of sodium hydrate, then fill the vessel to overflowing with warm dilute alcohol; carefully fit the cork so as to exclude the air. After the mixture has stood for some hours, a clear, yellow solution is obtained, the indigo-blue having been changed to indigo-white,  $C_{16}H_{11}N_2O_2$ . If now the clear solution be decanted and left exposed to the air, oxygen is taken up, indigo-blue is again formed and is deposited in crystals. Instead of grape sugar and alcohol, ferrous sulphate and warm water may be used in the preparation of indigo-blue; but in this case, the deposit will be amorphous.

Indigo-blue dissolves in concentrated sulphuric acid, espe-

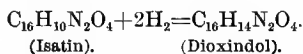
cially on being warmed, forming a deep blue solution of *indigotin-disulphonic acid* and *indigotinmonosulphonic acid*. On diluting this solution with water, the latter acid falls as a blue precipitate and may be removed by filtration. If a solution of indigo-blue in sulphuric acid be neutralized with sodic or potassic carbonate, a blue precipitate is formed. This precipitate is soluble in pure water, and forms the indigo-carmin sold in the shops.

If indigo-blue be boiled with water, and nitric acid be gradually added to the boiling mixture until all the blue color is destroyed, *isatin*,  $C_{16}H_{10}N_2O_4$ , is obtained, and forms, on cooling in beautiful prisms with a red lustre.

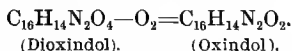
Indol has already been referred to as being the source of the normal odor of fæces and as being obtained from indigo (see page 67). But since an understanding of the physiology of the indigo-forming substances in the urine will depend upon our knowledge of the chemistry of indol, this subject demands more detailed consideration. Indol is obtained by the reduction of indigo. It has been shown above that isatin results from the oxidation of indigo-blue; this change is shown in the following equation:



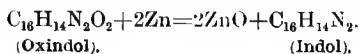
Now if an alkaline solution of isatin be treated with sodium amalgam, and the solution be evaporated, the sodium compound of *dioxindol* is formed, and deposited in glistening crystals. The isatin has been reduced:



If now dioxindol be farther treated with tin and hydrochloric acid, or with sodium-amalgam, farther reduction takes place and *oxindol* is formed:



If oxindol be heated with powdered zinc a farther reduction occurs, and *indol* remains as a final product in the reduction of indigo. All of the oxygen has been removed from the molecule:



It will be seen that each element in the molecule of indol is represented by an even number of atoms; consequently the formula for indol is generally written  $C_8H_7N$ . These formulæ do not represent theoretical changes, but those that have actually been made, and any chemist may thus obtain indol by the reduction of indigo.

§ 187. *Physiology*.—We are now ready to enter understandingly upon the study of the physiology of this subject. Whence arise the indigo-forming substances of the urine? The answer is, they arise from the oxidation of indol. If indol be injected subcutaneously it appears in the urine as indigogen. Then this question is asked, what is the source of the indol? It is the albuminous part of the food. In what part of the body and by the action of what agent or agents is the indol formed? It is formed in the intestines during pancreatic digestion, and is probably increased by fermentative changes. Kühne submitted albumen mixed with from eight to ten times its weight of caustic potash to dry distillation in metal retorts. The heat was gradually applied and increased to dull redness. He then redistilled the residue with water and extracted the remaining residue with ether. In the united distillates and extracts, he obtained a substance closely resembling indol. Later, Kühne and others have obtained pure indol by artificial pancreatic digestion of albumen. It is now a recognized fact that indol in small quantity results normally from digestion in the intestines. Consequently, we know the source of the indol, and know that when injected into the blood, it appears as indigogen in the urine.

§ 188. *Pathology*.—What has been said concerning the physiology of these coloring matters is substantiated by every fact which we know concerning their pathology. There is an excess of indigogen in the urine of those who suffer from obstructions of the intestines. The food retained in the intestines undergoes putrefaction, and thus increases the amount of indol that is absorbed. Jaffe found that ligating the small intestine of a dog increased the quantity of indigogen. In catarrh of the intestines and in diffuse peritonitis, the amount

of indigogen has been found increased. It is a notable fact that the first urine passed after a cholera collapse contains a great excess of indigogen. Thudichum gives the following very reasonable explanation of this fact: "In the course of the choleraic process, large quantities of albuminous matters in the muscles and organs lose their colloid state; and having in this process of liquefaction absorbed the necessary amount of heat, and thus produced the low temperature observed in all cases of collapse, pass into the blood, and from this into the intestinal canal. Here they are immediately subjected to a fermentative process, which resembles in many respects the kind of putrefaction, modified by pancreatic ferment, which we have described above."

It must be remembered that the color of the urine is no indication as to the quantity of indigogen present; thus, large quantities are not unfrequently detected in pale urines.

We thus see that indigo may be obtained from the urine, then reduced artificially to indol; then the indol may be injected subcutaneously and will again appear in the urine as indigogen, from which indigo and indol may again be obtained. Can any one study this subject, and then say, honestly, that the changes going on in the body are produced wholly by "vital force," and do not depend upon the laws of chemistry?

§ 189.—*Urobilin*,  $C_{32}H_{40}N_4O_7$ .—This coloring matter was first discovered by Jaffe, who named it urobilin; afterwards, Maly showed that it was identical with hydro-bilirubin and could be obtained by the action of sodium-amalgam on bilirubin (see p. 57). It is prepared from bilirubin by the following method: To bilirubin suspended in water, a piece of sodium-amalgam is added; the whole is allowed to stand exposed to an ordinary temperature for a short while, and then gently warmed on the water-bath. The supernatant fluid is then poured off from the mercury and treated with either hydrochloric or acetic acid. The acid precipitates the urobilin or hydrobilirubin as a reddish-brown powder, which may be collected, washed with water and dried.

Jaffe's method of preparing urobilin is as follows: Treat

the urine with basic acetate of lead; collect the precipitate which forms; wash it with water; dry it; then pulverize it and boil with spirits of wine, then add absolute alcohol acidified with sulphuric acid. This acidified alcohol dissolves the urobilin forming a wine-red solution which is separated from insoluble substances by filtration. The filtrate is treated with an excess of ammonium hydrate and again filtered. This filtrate is diluted with an equal volume of water, and then treated with zinc chloride as long as a reddish-brown precipitate falls. This precipitate is collected upon a filter and washed first with cold, and then with hot water, then dried. The dried precipitate is placed in a beaker and treated with alcohol acidified with sulphuric acid. This again dissolves the coloring matter, and the solution is filtered. To the filtrate add half its volume of chloroform, then an excess of water; shake well and remove the chloroform, which now contains the urobilin. The chloroform is washed well with water, and then the chloroform is evaporated, when urobilin remains. In the highly colored urine of fever, the urobilin may be precipitated by the direct addition of ammonium hydrate and zinc chloride.

Urobilin is a brownish-red, amorphous powder; soluble in alcohol, ether and chloroform, forming solutions which are red, brownish-red, or yellow according to the degree of concentration. The chloroform solution of urobilin, obtained from the urine, exhibits a marked green fluorescence; while the solutions of that obtained from bilirubin do not manifest this property until they have been rendered alkaline with ammonium hydrate, and then treated with a few drops of a solution of zinc chloride; after this treatment, the fluorescence is very marked. Urobilin is very sparingly soluble in water, freely soluble in alkalis, and in alcohol acidified with sulphuric acid. Spectroscopical examination of an acid solution of this coloring matter reveals a band, not very sharply defined, between b and F. In alkaline solutions, the band is more sharply defined and lies nearer b. If to an ammoniacal solution of urobilin, zinc chloride be added until the precipitate, which first appears, is redissolved, then this solution be examined

with the spectroscope, the above mentioned band will be much more sharply defined. \*

Urobilin readily combines with alkalis forming compounds which are soluble in water and in an excess of the alkali. It is precipitated from neutral solutions on the addition of zinc chloride. This salt is reddish-brown in color, and is freely soluble in ammonium hydrate. Thudichum claims that urobilin is a mixture of several substances and that it is not identical with hydrobilirubin; but Hoppe-Seyler thinks that the *uromelanin*, *paramelanin*, *omicholin*, *omicholic acid*, etc., of Thudichum need further investigation.\*

§ 190. *Physiology*.—Maly thinks that urobilin results from the reduction of bilirubin and that this reduction takes place in the intestinal canal. Hoppe-Seyler has prepared a substance, which has the properties of this coloring matter, by the action of hydrochloric acid and tin on hæmatin. This author also thinks that urobilin, as such, is not contained in normal urine, but that it is formed during the process of separation by the oxidation of other substances. It seems evident that this coloring matter results from that of the blood and represents the disintegration of the red corpuscle; normally it first passes from the blood into the bile and there exists as bilirubin and other bile-pigments.

§ 191. *Pathology*.—Urobilin is increased in fevers and the amount excreted, in a given time, varies with the heat of the fever. In the highly colored urine of fever, this coloring matter may often be detected by spectroscopic examination of the filtered, acid urine; or by adding an excess of ammonium hydrate, filtering, adding zinc chloride and then applying the spectroscopic test; by precipitating the urine with the basic acetate of lead, washing this precipitate with water, dissolving it in alcohol acidified with sulphuric acid, rendering alkaline with ammonium hydrate, adding zinc chloride and examining with the spectroscope. (Hoppe Seyler).

§ 192. *Uroerythrin* is the name given by Heller to the coloring matter which so often causes a deposit of urates to have a

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\* Handbuch, S. 217.

pink or reddish tint. It is abundant in cases of acute rheumatism and is identical with the murexid of Prout and with the purpurin of Golding-Bird.

GRAPE SUGAR,  $\text{—C}_6\text{H}_{12}\text{O}_6$ .

§ 193. To the chemist, the term sugar, is somewhat indefinite, and indicates the class rather than the individual. The sugar, which is a constant constituent of the blood, is identical with grape sugar. It is prepared on a large scale by boiling starch for several hours with dilute sulphuric acid, then neutralizing the acid with carbonate of lime, filtering and evaporating, when the grape sugar crystallizes. It may also be obtained in a very pure state from diabetic urine by the following process: Concentrate the urine to a syrup on the water-bath; allow the syrup to stand for several days, when the solid constituents will have crystallized; wash the crystalline mass with a little cold alcohol, which dissolves the urea; treat the residue with hot alcohol; filter while hot, and allow to stand, when the sugar crystallizes in warty granules. These may be redissolved in hot alcohol, filtered, and allowed to recrystallize.

Sometimes four-sided prisms can be obtained, but generally only granules form. Grape sugar is slowly soluble in water, and on being dissolved in water it loses its property of crystallization; for if an aqueous solution be evaporated, only an amorphous mass remains. The crystals of grape sugar contain water, and are represented by the formula,  $\text{C}_6\text{H}_{12}\text{O}_6 + \text{H}_2\text{O}$ . If sodium chloride be present, it combines with the sugar, forming large, six-sided, double pyramids, which have the composition represented by the formula,  $2\text{C}_6\text{H}_{12}\text{O}_6 + \text{NaCl} + \text{H}_2\text{O}$ .

(1) *Trommer's Test.*—When grape sugar is boiled with an alkaline solution of a cupric salt, the copper is reduced and deposited as a red or yellowish precipitate of the suboxide,  $\text{Cu}_2\text{O}$ . Upon this fact depends Trommer's test for sugar, which is applied as follows: To a solution of grape sugar, or to diabetic urine, add a few drops of a dilute solution of copper sulphate, render the mixture alkaline with potassium or sodium hydrate, and heat, when the suboxide of copper is

precipitated. If a solution of grape sugar in pure water be used for this test, the precipitate will be colored red, while if the test be applied to diabetic urine, the color of the precipitate will vary between a red and a yellow, depending upon the amount and kind of organic coloring matters present. Instead of applying the solution of copper and the alkali separately in this test we now use Fehling's solution. This consists of a solution of copper sulphate mixed with a solution of Rochelle salts (tartrate of potassium and sodium) in sodium hydrate\*. The sodium hydrate added to a solution of copper sulphate would throw down a precipitate of the black oxide,  $\text{Cu O}$ ; consequently the alkaline tartrate is added in order to hold the black oxide in solution.

In the application of Trommer's test to the urine in searching for sugar, some caution must be used. In the first place it must be remembered that the disappearance of the blue color of the Fehling solution alone is no proof of the presence of sugar. The blue is caused to disappear by the action of the alkali upon the organic matter present. Not only must the blue color disappear, but there must be a distinct precipitate of the suboxide of copper. Now this precipitate is to be recognized by its color; because, since Fehling's solution is alkaline, it will throw down a dirty white precipitate of phosphates, which is to be distinguished from that caused by sugar.

It is also quite essential to regard the amount of Fehling's solution that is added to the fluid under examination for sugar. If too much of this test fluid be added, the blue color of the excess may hide from view a small quantity of the suboxide which may be formed. If too small a quantity of Fehling's solution be added, the small amount of suboxide, which may be formed, may be hidden by the color of the urine or other fluid under examination. If there be albumen in the fluid, it must be removed before the application of Trommer's test for sugar. If the fluid be already acid, and contains but little albumen, this is removed by heat and filtration. If the fluid

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\*The method of preparing Fehling's solution is given under the subject of the Quantitative Estimation of Sugar in Urine.



be not acid it should be rendered so by the addition of acetic acid (an excess of the acid is to be avoided), then heated and filtered. If this is done the filtrate should be rendered alkaline before the application of the test for sugar; and it is well to remark here that any strongly-acid fluid should be neutralized or rendered alkaline, before the application of Trommer's test. If there be a large quantity of albumen in the fluid to be tested for sugar, the albumen is best removed by adding to the solution an equal weight of crystals of sodium sulphate, boiling and filtering. Thus, in testing blood for sugar, a weighed portion of the blood should be mixed with an equal weight of crystallized sodium sulphate, then a hot saturated solution of sodium sulphate is added; the mixture is then boiled and filtered, when, if sugar be present, it may be found in the filtrate on the application of Trommer's test.

Roberts advises the following method of applying Trommer's test: Pour Fehling's solution to the depth of three-fourths of an inch into a test tube; boil, and then add a drop or two of the urine. In a few seconds the mixture will suddenly become yellow, if the specimen be ordinary diabetic urine; but if only a small amount of sugar be present more urine, but not more than the Fehling solution taken, must be added. In the latter case, the mixture must again be boiled and allowed to cool slowly.

(2) *Moore's Test*.—If a solution of grape sugar be boiled with sodium or potassium hydrate, the sugar is decomposed, while the solution becomes colored brown. Upon this reaction depends Moore's test for sugar. This test is best applied as follows: To a small test tube about one-half or two-thirds full of the fluid under examination, add sufficient sodium or potassium hydrate to render strongly alkaline; holding the test tube by the bottom, heat the upper part of the fluid in the flame; the sugar contained in the heated portion will be decomposed and produce a brown coloration. The advantage to be derived from heating the upper portion of the fluid is that decomposition of the sugar takes place only in the heated portion and the contrast between this and the unchanged portion renders the color much more distinct. If but little sugar be present, the color

produced on the application of this test will be a light yellow; this deepens as the amount of sugar is increased and may be quite black. Certain albuminous urines containing no sugar respond to this test.

(3) *Bættcher's test*.—To a solution of grape sugar or to some diabetic urine, add an equal volume of a concentrated solution of sodium carbonate, then add a small piece of the basic nitrate of bismuth and boil the mixture. The bismuth is reduced to a suboxide, the reduction being indicated by the change of the color of the bismuth to gray, and then, if sufficient sugar be present, to black. If only traces of sugar be present, but little bismuth must be added. Instead of the basic nitrate of bismuth, a preparation obtained by the following process may be used: To nitrate of bismuth, add a large excess of potassium hydrate; collect the precipitate which forms, and add sufficient tartaric acid to dissolve it. If a drop of this solution be boiled with one of grape sugar, the bismuth falls as a black precipitate.

(4) *Mulder's test*.—Render a solution of indigo alkaline by the addition of sodium carbonate; boil this with a solution of grape sugar, when the blue color disappears and the fluid becomes yellow. If but little sugar be present, the solution will become purple instead of yellow, and even when much sugar is present, the purple may be noticed as a transitory color. Now if the yellow fluid be shaken with free access of air, the original blue color again appears. This test is not very suitable for application to the urine, unless considerable sugar be present.

(5) *Fermentation test*.—If a test tube be filled to overflowing, with a feebly acid solution of grape sugar, a small piece of German yeast be added, and the tube closed with a tightly fitting cork, or a rubber stopper, which has a small glass tube passing through it, with one end extending down into the solution and the other bent at right angles, vinous fermentation will take place and the carbonic acid gas will rise to the top of the test tube and, by its pressure, will force some of the solution out through the small tube. The test tube should be left in a warm place for several hours, when the stopper may be removed and the carbonic acid tested with a lighted match. Some specimens

of yeast give off from themselves small quantities of carbonic acid gas, therefore it is always well to prepare a companion tube containing yeast and distilled water.

§ 194. *Physiology*.—Sugar is present in the blood, chyle and lymph; whether it be a constituent of normal urine or not, is a question which has long been under investigation by physiologists, and seems to be now quite positively decided in favor of the affirmative by the experiments of Pavy. This untiring investigator has labored in this field for years and has done much in bringing out new facts in regard to the physiology and pathology of sugar. Pavy's method of testing normal urine for sugar may be expressed as follows: To some normal urine (100 c. c. or more), add the normal acetate of lead in an excess. This throws down a heavy precipitate consisting of the chloride, sulphate, phosphate, urate and probably other constituents. This precipitate is removed by filtration. To the clear filtrate, which contains an excess of lead acetate, ammonium hydrate is added. Another copious precipitate falls, and among other things, contains the sugar combined with the oxide of lead. Sugar does not combine with the oxide of lead in an acid solution, and for this reason, it escapes precipitation on the first addition of lead acetate to the urine. The precipitate produced with the lead acetate and ammonium hydrate is washed with hot water, at first by decantation and is then collected upon a filter and the washing is continued until the filtrate is no longer found alkaline when tested with red litmus paper. However, excessive washing is to be avoided, because the sugar may be removed. (Pavy). The washed precipitate is now suspended in a little distilled water and treated with a current of hydrosulphuric acid gas for some hours. The precipitated lead sulphide is removed by filtration, and the filtrate, containing the sugar, is then heated until the hydrosulphuric acid is driven off. The fluid is now concentrated to a small volume, either on the water-bath or in vacuo. This concentrated fluid contains the sugar which will respond to Trommer's test or to any of the other tests as already given.\*

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\* For Pavy's description of this test, see London Lancet, March 30, 1878.

Not only has Dr. Pavy obtained the qualitative test for sugar, in normal urine, but he has estimated the quantity excreted, and found that it varies from .09 to .5 of a part per 1000. When a quantity of sugar greater than .5 of a part per 1000 is present in the urine, it can generally be detected by the ordinary applications of the tests and then the person is said to have diabetes.

The old doctrine of Bernard is that the liver is a sugar-forming organ; the experiments of Pavy go to prove that it is not a sugar-forming, but is a sugar-assimilating organ. According to the former, the office of the liver is to furnish the blood with sugar; according to the latter, the office of the liver, in this respect, is to prevent the passage of the sugar into the blood. It was formerly supposed that the sugar was formed in the liver, then was carried out with the blood, and was oxidized during its passage through the lungs. But analyses of the blood, taken from both sides of the heart, showed that the sugar was not perceptibly lessened during the passage of the blood through the lungs. It was then supposed that the sugar was consumed or absorbed during the passage of the blood through the systemic capillaries. Indeed, Bernard made quite a number of analyses of arterial and venous blood, which seemed to prove that the former contained more sugar than the latter (*Lecons sur le Diabete*). But Pavy, employing another process (and evidently a much more reliable one) of estimating the amount of sugar, and using more care in collecting the blood, has shown that the difference between the amount of sugar in arterial and that in venous blood is very small. Pavy found as the average for eleven estimations (four made upon the arterial and venous blood collected simultaneously from the dog immediately after death; and seven made upon the arterial and venous blood collected simultaneously from the dog during life) that the arterial blood contained 0.941 of a part of sugar, and the venous blood, 0.938 of a part of sugar per 1000 of blood.

From these experiments, it seems quite evident that there is no considerable destruction of sugar in the systemic capillaries. Then the question arises, what does become of the sugar of the

food? Some of it probably passes through the absorbents into the thoracic duct and then into the general circulation; but the greater part of the sugar is absorbed into the portal system and carried to the liver. After reaching this organ, probably more of the sugar passes on unchanged into the general circulation; but the greater part of the sugar is transformed into glycogen. Then the question arises, what becomes of the glycogen that is formed in the liver from the carbohydrates and albumen of the food? The old glycogenic theory held that this glycogen of the liver was transformed into sugar, which passed out in the blood. But Pavy and Tscherinoff have shown that the blood of the hepatic vein contains no more sugar than does that of the portal vein or that of the heart. Moreover, if the glycogen is transformed into sugar, what becomes of the sugar thus formed? We have already seen that the sugar is not oxidized either in the lungs or in the systemic capillaries. In fact it is not known how the blood can oxidize sugar. Then to answer the question, what is the fate of the glycogen? It may be answered that the destination of the glycogen is not positively known. It evidently serves as a reserve which is stored up during digestion and is afterwards called upon, during the hours of fasting, to yield its supply of force. It is also evident that the ultimate products of the oxidation of the sugar of the food or of the glycogen of the liver are carbonic acid and water. The most plausible theory with regard to the fate of the glycogen, is that it is converted into fat. Animals fed upon starchy food fatten more rapidly than when this kind of food is withheld; but how the glycogen is transformed into fat is not known.

It must be borne in mind that the idea of the physiology of sugar, as given above, does not invalidate the statement that the carbohydrates are valuable force-producing foods. The final products of this kind of food are water and carbonic acid; and the transformation of the sugar into glycogen and of the latter into fat (if such a transformation does take place) does not lessen the amount of force furnished by the sugar.

§ 195. *Pathology.*—Pavy teaches that whenever the glycogenic function of the liver, as taught by Bernard, is established,

diabetes results. In health, the liver prevents the larger portion of the sugar reaching the blood and thus prevents diabetes. If the sugar reaches the blood, as sugar, it cannot be oxidized and consequently is excreted in the urine. Therefore if the liver fails to arrest the sugar and to transform it into glycogen, the former passes on, unchanged, into the blood and is carried to every part of the body and a proportional amount will be excreted in the urine. Again, it is well known that from post mortem changes in the substance of the liver, a ferment is generated and converts the glycogen into sugar. This ferment, or a similar one, may be generated in certain diseased states and may cause the conversion of the glycogen into sugar. Pavy found that diabetes could be produced by surcharging the blood with oxygen by means of artificial respiration. It is also well known that the inhalation of carbonic oxide, ( $\text{CO}$ ), causes diabetes. Now it is supposed that either the carbonic oxide gas, itself, or the carbonic oxide-hæmoglobin, which results from the combination of the gas with the coloring matter of the blood, abnormally stimulates the liver, and causes the conversion of the glycogen into sugar.

In diabetes mellitus, the amount of urine is increased; but the increase is seldom so great as that of diabetes insipidus. The specific gravity is high, unless there be albumen present. In a case of diabetes mellitus co-existing with albuminuria, I found the specific gravity as low as 1010. In this disease, the total quantity of urea for the 24 hours is greatly increased; for as has been stated, the escape of the sugar from the body, without yielding any force, causes a greater consumption of the nitrogenous food and tissues. The amount of urinary water may be so great as to cause a deficiency of urea in proportion to the water; but the total quantity for the 24 hours will be excessive.

The high specific gravity is due to the presence of the sugar, and in a marked case of diabetes mellitus, the density is seldom less than 1030 and may be as high as 1060. The total quantity of sulphates, phosphates, and chlorides is often increased. This is also due to the excessive destruction of nitrogenous food

and tissue. The amount of sugar varies from a barely perceptible trace to 600 grams for the 24 hours. Remember that sugar is always present in the urine, but that it is only when the quantity is sufficient to be detected by the ordinary tests that it is abnormal. The excretion of sugar in diabetes varies with the kind of food, being increased when much starchy or saccharine food is taken; but the sugar does not disappear from the urine when the food consists entirely of albuminous substances. However, the patient who excretes 10 grams of sugar per day, when living entirely upon animal food, is to be regarded as in a more serious condition than he who, while living upon starchy food, excretes 100 grams of sugar per day.

## QUANTITATIVE ANALYSIS OF URINE.

### ESTIMATION OF UREA.

§ 196. (1) *By Liebig's Method.*—This depends upon the fact, that in neutral and alkaline solutions, mercuric nitrate precipitates urea, and that as soon as an excess of the mercury solution has been added, the excess will be shown by placing a drop of the mixture on a glass slide with a drop of sodium carbonate solution, when a yellow coloration will be produced.

*Preparation of the Mercury Solution.*—Dissolve 77.2 grams of pure red oxide of mercury, or 71.5 grams of the metal, in strong nitric acid. Apply heat and add more acid, if necessary, until all the mercury has been converted into *mercuric* nitrate, which will be indicated by the failure to produce a precipitate in some of the solution diluted with water on the addition of a solution of sodium chloride; because *mercurous* chloride is insoluble, while *mercuric* chloride is soluble. Then drive off excess of acid and dilute to 1000 c. c. If on diluting, any precipitate should appear, nitric acid must be added drop by drop, sufficient to dissolve the precipitate, but avoiding any excess. Each c. c. of this solution will precipitate .01 gram of urea.

*Preparation of the Baryta Mixture.*—Before the urea can be precipitated from the urine by the mercury solution, the sulphates and phosphates must be removed. This can best be

done by precipitating them with a mixture of two volumes of cold saturated solution of barium hydrate, and one volume of ditto barium nitrate. This is known as the "baryta mixture."

*Application to the Urine.*—To 20 c. c. of urine add 10 c. c. of the baryta mixture; filter; to 15 c. c. of the filtrate in a small beaker, add the mercuric nitrate solution, slowly from the burette, until a drop from the beaker placed on a glass slide, with a drop of a solution of sodium carbonate, turns from a white to a yellow color. Read off from the burette the amount of the mercury solution used. Each c. c. of this will indicate .01 of a gram of urea in every 10 c. c. of urine. From this, the total amount in the twenty-four hours' urine may be calculated.

In some cases, certain errors arise from this method of estimating urea. I will not enter into detail, but will mention some of the more important errors with the best methods of avoid them.

If the urine contains more than 10 grams of sodium chloride in every 1000 c. c., 2 c. c. must be deducted from the number of c. c. of mercury solution used for the 10 c. c. of urine; because *that* much of the solution would be taken up by the chlorides.

If the urine contains albumen, this must be removed by heat, acetic acid and filtration.

If part of the urea has been decomposed into ammonium carbonate, this will interfere with the estimation of the remaining urea, and must be disposed of by evaporating to dryness, when the ammonia will be driven off and the residue may be redissolved in water: and the urea which it contains estimated as before. The alkalinity caused by the ammonia may be estimated with a normal acid solution, and the amount of urea calculated from this.

§ 197. (2) *Estimation of Urea by Conversion into Nitrogen Gas.*—Bearing in mind the objections to Liebig's method, Russell and West invented an apparatus for decomposing the urea and measuring the liberated nitrogen. This depends upon the fact that if a solution of urea be treated with an alkaline solution of sodium hypochlorite, or hypobromite, urea is at



once decomposed and nitrogen gas given off. It only remains to construct an apparatus suitable for this reaction. Many modifications have been proposed, but one of the simplest and cheapest is described by Sutton, as follows: "The tube for decomposing the urine is about nine inches long, and about half an inch inside diameter; at two inches from its closed end an elongated bulb is blown, leaving an orifice at its neck of three-eighths of an inch in diameter; the bulb should hold about twelve c. c.; the mouth of this tube is fixed into the bottom of a tin tray about one and three-quarter inches deep, which acts as a pneumatic trough; the tray is supported on legs long enough to allow of a small spirit lamp being held under the bulb tube; the measuring tube is graduated so that the amount of gas read off expresses at once what may be called the percentage amount of urea in the urine experimented upon, *i. e.*, the number of grams in 100 c. c., five c. c. being the quantity of urine taken in each case."

The *hypobromite* solution is best made by dissolving 100 grams of caustic soda in 250 c. c. of water and adding to this, when cool, twenty-five c. c. of bromine. This solution must be kept in the dark, and will become unfit for use within two or three weeks under any circumstances. If only a few estimations are to be made at a time, it would be better not to make the full quantity as given above, but to take proportional parts.

*Application to the Urine.*—Pour five c. c. of the urine into the bulb and fill up to the top of the constriction with water, in order to exclude all air; but the water must not extend much above the constiction. Take a solid glass rod as long as the bulb tube, with a piece of thin rubber tubing drawn over one end, which should fit tightly into the upper part of this constriction. Place this tube, which acts as a stopper, in position, and fill the upper part of the bulb tube with the the hypobromous solution. Fill the trough half full of water. Fill the measuring tube with water, and with the thumb over its open end, invert it in the trough. If any air rises in this tube, again fill with water and invert, repeating, if necessary, until no air remains in the tube after inversion. Remove the stopper and

place the open end of the measuring tube over the bulb tube. As soon as the stopper is removed, the hypobromite passes down into the bulb, coming in contact with the urine and liberating the nitrogen, which rises into the measuring tube, from which the per cent. of urea is read off.

If the urine is albuminous, the albumen should be removed by heat and acetic acid, as given under Liebig's method. The albumen effects the operation only so far as it takes a longer time for the bubbles of gas to subside, so that the per cent. may be read off.

If the urine under examination contains a great excess of urea, so that the measuring tube will not hold all the gas liberated, dilute a certain quantity of the urine with an equal bulk of water and use five c.c. of this solution. In this case the amount of gas as read off must be doubled in order to have the correct percentage.

#### ESTIMATION OF CHLORIDES.

(CALCULATED AS SODIUM CHLORIDE).

§ 198. *Liebig's Method.*—This depends upon the fact that, if a solution of mercuric nitrate be added to one of sodium chloride, mercuric chloride is formed and the solution remains clear. Now if urea be present it will precipitate the mercury as soon as all the chlorides have been taken up. Consequently, in estimating the chlorides in the urine with mercuric nitrate, the process is complete as soon as a permanent cloudiness appears.

*Standard Solution of Mercuric Nitrate.*—It is necessary that this solution should be as pure as possible, and especially that it should not contain any silver or lead, as these would precipitate the chlorides and interfere with the test.

Take 18.42 grams of pure red oxide of mercury, dissolve in nitric acid, converting it all into the *mercuric* salt as under urea. Any excess of acid must be avoided. Dilute to one liter. Each c.c. of this solution will take up .01 of a gram of sodium chloride.

*Application to the Urine.*—To twenty c.c. of urine add ten

c.c. of the baryta mixture (same as used in estimating urea); filter; to fifteen c. c. of the filtrate neutralized or rendered feebly acid with nitric acid, add slowly from the burette the mercuric nitrate solution, until a permanent cloudiness appears. Read off from the burette the amount of this solution used. Each c. c. will indicate .01 of a gram of sodium chloride in each ten c. c. of the urine; from which, the amount in the twenty-four hours' urine can be calculated.

Example: Suppose that it requires six c.c. of the mercurial solution to produce the cloudiness, and that 1200 c. c. were passed during the twenty-four hours; then the total amount of sodium chloride would be found by the following proportion: 10 c. c. : 1200 c. c. : : .06 grams : x—7.20 grams.

#### ESTIMATION OF SULPHURIC ACID.

(ESTIMATED AS  $\text{SO}_3$ ).

§ 199. *Standard Barium Chloride*.—Dissolve 30.5 grams of pure crystallized barium chloride in some distilled water and dilute to one liter. Each c. c. of this solution will equal .01 gram of  $\text{SO}_3$ . A dilute solution of sodium or magnesium sulphate will also be required.

*Application*.—Fifty c. c. of clear urine are poured into a beaker, acidified with hydrochloric acid, and heated on the sand-bath. As soon as the solution boils the lamp is removed and the barium chloride is allowed to flow slowly from the burette into the beaker, and it must continue to flow as long as the precipitate is seen to increase. The precipitate is allowed to subside, then more of the barium chloride is added, and this process repeated until no farther precipitate is produced. Much time and labor will be saved by filtering a few drops of the solution every now and then, and allowing these to fall into a test tube containing some of the dilute sodium or magnesium sulphate. As soon as an excess of barium chloride has been added a precipitate will appear in the test tube. Read off from the burette the amount of barium chloride used; each c. c. of which will indicate .01 of a gram of  $\text{SO}_3$  in each 50 c. c. of urine, and from this the total amount may be calculated.

## ESTIMATION OF PHOSPHORIC ACID.

(ESTIMATED AS  $P_2O_5$ ).

§ 200. *By Uranium Acetate.*—This method is based upon the fact that when a solution of uranium acetate is added to a solution containing soluble phosphates, sodium acetate and free acetic acid, all the phosphoric acid will be precipitated as uranium phosphate. This precipitate is of a light yellow color, insoluble in acetic, but soluble in hydrochloric acid. The point of completion of the reaction may be ascertained by placing a drop of the yellow mixture upon a piece of filter paper, which has previously been moistened with potassium ferro-cyanide and dried. As soon as there is the slightest excess of the uranium acetate, the paper will be stained *brown*, due to the formation of uranium ferro-cyanide. The following solutions will be needed:

(1) *Solution of Potassium Ferro-cyanide*, about one part of the salt to twenty parts of water. The test papers are to be moistened with the solution and dried; they may be kept for months and still give the color on the application of a drop of dilute uranium acetate.

(2) *Solution of Sodium Acetate* is prepared by dissolving 100 grams of sodium acetate in distilled water, diluting to 900 c. c., and then adding 100 c. c. of acetic acid.

(3) *Standard Solution of Disodic Hydric Phosphate*, made by dissolving 50.4 grams of the crystallized salt in water and diluting to one liter. Each c. c. of this solution contains .01 of a gram of  $P_2O_5$ .

(4) *Uranium Acetate.*—Since this cannot be obtained sufficiently pure to be weighed out and used directly, we make a solution of it of indefinite strength and standardize it with the other solutions.

It has been found best to make the solution of uranium acetate of such a strength that each c. c. will precipitate .005 of a gram of  $P_2O_5$ . Now each c. c. of the sodium phosphate solution contains .01 of a gram of  $P_2O_5$ . Consequently, every 2 c. c. of the uranium acetate should be made equal to every 1 c. c. of the sodium phosphate: or upon adding 20 c. c. of the ura-

nium acetate to 10 c. c. of the sodium phosphate, and then touching the paper which has been moistened with the potassium ferro-cyanide, with a drop of the mixture, we should just get the brown color.

Put 10 c. c. of the sodium phosphate with 5 c. c. of the sodium acetate solution into a beaker. To this, add slowly from the burette the uranium acetate, testing, occasionally, for the color on the paper. Suppose that on the addition of 8 c. c. from the burette, the color is obtained, then 8 c. c. of the uranium acetate are as strong as 20 c. c. should be, and for every 8 c. c. of the uranium solution that we have, 12 c. c. of water should be added. If it should require more than 20 c. c. to produce the color, the uranium solution must be concentrated by evaporation or more of the solid salt added. The solution has now been graduated.

*Application.*—Fifty c. c. of clear urine, with 5 c. c. of the sodium acetate solution, are poured into a beaker and heated; to this, the uranic acetate is slowly added from the burette. The mixture is constantly stirred with a glass rod, which should be applied frequently to the test paper. As soon as the brown color is obtained the process is complete. Read off from the burette the amount of uranium acetate solution used. Each c. c. will indicate .005 of a gram in every 50 c. c. of urine.

#### ESTIMATION OF PHOSPHORIC ACID COMBINED WITH EARTHY BASES.

§ 201. The method just given determines the total amount of phosphoric acid, but the physician often desires to know the amount of phosphoric acid existing as earthy phosphates. To 100 c. c. of clear urine, add ammonium to a slight alkaline reaction; set aside for 12 hours. At the expiration of this time, the earthy phosphates will have subsided; the clear fluid is decanted through a filter, the phosphates collected on the same filter, and washed with distilled water, containing a little ammonium hydrate; then dissolved in acetic acid; the solution is diluted, sodium acetate added, and the phosphoric acid estimated with the standard solution of uranium acetate. Each c. c. of the uranium acetate used will represent .005 of a gram

of phosphoric acid in each 100 c. c. of urine; from this, the amount of phosphoric acid existing in combination with the earthy bases, in the total urine for the 24 hours, may be calculated. This subtracted from the total amount of phosphoric acid in the urine will give the amount of phosphoric acid in combination with the alkaline bases.

#### ESTIMATION OF CALCIUM AND MAGNESIUM.

§ 202. To 200 c. c. of urine add sufficient ammonium hydrate to produce a strongly alkaline reaction. Allow this to stand for some time and then collect the precipitate, which has formed, and which consists of the earthy phosphates, upon a filter. Dissolve this precipitate in acetic acid. To this solution, add a solution of ammonium oxalate, which throws down the calcium as an oxalate; while the magnesium remains in solution. Collect the calcium oxalate upon a filter (reserving the filtrate for the estimation of magnesium). Dissolve the calcium oxalate in dilute hot hydrochloric acid. To this solution, add a few drops of dilute sulphuric acid and then alcohol in large excess. The precipitated calcium sulphate is collected upon a filter (the filtrate being further tested by the farther addition of dilute sulphuric acid and alcohol to insure the precipitation of all the calcium) dried, ignited and weighed. This gives the weight of  $\text{CaSO}_4$  obtainable from 200 c. c. of urine; from this, the amount of calcium, in the 200 c. c. and then in the total urine for the 24 hours, may be calculated.

To the filtrate from the calcium oxalate, add ammonium hydrate to a strongly alkaline reaction, when the magnesium is thrown down as ammonio-magnesium phosphate. This precipitate is allowed to subside, which it readily does. It is then washed by decantation with water containing a little ammonium hydrate, transferred to a platinum dish, heated to redness, cooled and weighed as magnesium pyrophosphate,  $\text{Mg}_2\text{P}_2\text{O}_7$ . From this, the amount of magnesium in the 200 c. c. and in the 24 hours' urine may be calculated.

#### ESTIMATION OF URIC ACID.

§ 203. The volumetric method of estimating uric acid is open to so many objections and is, consequently, so unreliable

in any but the most experienced hands, that the gravimetric only will be given here.

To 200 c. c. of urine in a beaker, add 10 c. c. of nitric acid; mix well, cover with a piece of glass, and set in a cool place for twenty-four hours; at the end of this time, uric acid crystals will be observed on the bottom and sides of the beaker. Decant the supernatant fluid through a filter paper which has been, previously, dried and weighed; or through tarred filter papers; collect the crystals on the same filter, dry at  $100^{\circ}$  and weigh. The difference between the weight of the paper alone, and that of the paper with the crystals, will be the amount of uric acid in 200 c. c. of urine. More or less coloring matter adheres to the crystals and influences the weight, causing a slight error.

#### ESTIMATION OF FREE ACIDS.

§ 204. The acidity of the urine is, without doubt, due to several substances, among which may be mentioned acid phosphate of sodium, lactic, kryptophanic, and other organic acids. This estimation is made with a solution of caustic soda, which has been graduated so as to just neutralize a standard solution of oxalic acid of 10 grams of the pure crystallized acid, dissolved in water and diluted to one liter.

*Application.*—100 c. c. of urine are poured into a beaker and the standard alkali allowed to fall into this slowly, until a drop of the mixture, taken up with a fine glass rod or a feather and streaked across some delicate blue litmus paper, produces no change of color. The amount of the alkali is read off and the degree of acidity is registered as being equal to so much oxalic acid. Each c. c. of the alkali used is equivalent to .01 of a gram of oxalic acid.

#### ESTIMATION OF SUGAR.

§ 205. The most common method of estimating sugar is with a solution of copper sulphate, and is based on the fact, that if this salt be heated with a solution of tartrate of potassium and sodium in sodium hydrate, no reduction occurs; but as soon as some grape sugar is added to the heated mixture, the copper is reduced to the suboxide which is deposited as a red or yellow precipitate. In pure water, the precipitate would always be

red, but in the urine it has a yellow color. Many different preparations of copper for this test have been proposed, but the best, and the one almost exclusively used is Fehling's solution, which is prepared as follows:

(1) Weigh out 34.65 grams of pure crystallized copper sulphate, pulverize in a mortar and dissolve in 200 c. c. of distilled water.

(2) Dissolve sodium hydrate in 500 c. c. of water until the solution has a sp. g. of 1.14; then dissolve, in this solution, 173 grams of crystallized Rochelle salts.

Gradually mix the two solutions, stirring with a glass rod. The mixture will have a deep blue color, and must be diluted to one liter; when 10 c. c. of it will just be decolorized by .05 of a gram of grape sugar.

*Application.*—Measure into a clean porcelain dish 10 c. c. of Fehling's solution with an equal bulk of water; 10 c. c. of the urine are diluted to 100 c. c. with distilled water, and the burette filled with this solution, which is allowed to fall into the *boiling* dish of Fehling's solution until the blue color is entirely destroyed. Read off from the burette the amount used, one-tenth of which is urine, and contains .05 of a gram of grape sugar.

For Knapp's method of estimating sugar with mercuric cyanide, see Sutton's "Volumetric Analysis." The only advantage that the mercuric cyanide has over Fehling's solution is that it will keep longer without deterioration. If the Fehling's solution is not fresh, it is always best to boil it alone for some time, and if the red or yellow precipitate is not thrown down, it is still fit for use.

*Roberts' Differential Density Method.*—When diabetic urine is fermented by the addition of yeast, the sugar is destroyed with the formation of carbonic acid and alcohol. This lessens the specific gravity of the urine and the more sugar originally present the greater will be the decrease in the density after fermentation. Dr. Roberts found that this decrease is constant, and is one degree for every grain of sugar per ounce of urine. He recommends the following method: Pour about four ounces of the urine into a twelve ounce bottle, add a piece of German



yeast the size of a walnut and stop with a nicked cork so as to allow the escape of gas. Fill a four ounce bottle with the same urine without any yeast and cork tightly. Leave the two bottles side by side in a warm place for about twenty-four hours. Then allow both specimens to stand in a cool place for some time. Decant the fermented urine and with a urinometer take the specific gravity of each specimen. The difference will represent the "density lost" and the number of grains of sugar per ounce of urine. This method is sufficiently accurate for clinical purposes.

#### ESTIMATION OF ALBUMEN.

§ 206. *By weight.*—Pour 50 c. c. of distilled water into an evaporating dish, acidify with a drop or two, not more, of acetic acid, place on the sand-bath and boil. To this, while boiling, add slowly 50 c. c. of the clear filtered urine. While adding this, test, frequently, the contents of the dish, with the blue litmus paper, and if the reaction is not acid, add a drop or two of acetic acid, always avoiding any excess. The albumen will be coagulated and must be collected upon a filter, which has been previously dried and weighed; the precipitated albumen is washed on the filter with distilled water (until, on evaporating a few drops of the filtrate to dryness, no residue is left) dried at 110° and weighed. Deducting from this, the weight of the filter paper, we have the amount of albumen in 50 c. c. of the urine, and from this the amount in the twenty-four hours urine may be calculated.

§ 207. *Clinical Method.*—The physician often does not care to know how many grams of albumen his patient passes in twenty-four hours; but he is anxious to know whether the daily amount is on the increase or decrease. He desires to find out the proportion between the amount passed on one day and that passed on another. For this purpose the following method is applicable.

Dilute the twenty-four hours' urine to 3000 c. c. Precipitate a definite part of this, in a test tube, with heat and a few drops of nitric acid; allow the coagulum to completely subside, and mark the tube so as to indicate the bulk of albumen, or if the

next estimation is to be made within a few days, leave the albumen in the tube with the supernatant fluid. When the next estimation is made, proceed exactly in the same manner; diluting the twenty-four hours' urine to the same quantity; taking the same definite part of this and precipitating in the same tube, or in one of the same size. By a comparison of the bulk of coagula obtained in the two instances, it is easily ascertained whether the albumen has increased or decreased in amount.

#### THE RESULTS OF A QUANTITATIVE ANALYSIS.

§ 208. After a quantitative analysis of a specimen of urine has been made, it is desirable to present the results in some compact form. For the analysis, the 24 hours' urine should be obtained and measured as has been directed (see p. 185 et seq.). The specific gravity of the mixed urine should be ascertained and the total weight of the urine and of the solids calculated according to the rules given on pages 205 and 206. Then each of the constituents should be estimated.

The results of the analysis may be represented as in the table on the following page. It is well to represent the quantity of urine in terms of both the French and English measures. In this, 30 c. c. are considered as equivalent to one fluid ounce; this, it is true, is not the exact equivalent, but since the amount in cubic centimeters is exact, that in ounces is only used as an indication of the approximate equivalent in the English measure. The total weight of the urine, solids and each constituent is also represented in both grams and grains; in this, it is considered that 15.43 grains are equivalent to 1 gram.

A person weighing 200 lbs. will probably consume more nitrogenous food and excrete more urea than one, in the same state of health, who weighs but 100 lbs. Consequently, one column is given showing the proportion in grams per kilogram, and another showing the proportion in grains per pound of the body weight. One kilogram is considered as the equivalent of 2.2 pounds.

In the sample table for exhibiting the results of a quantitative analysis, all of the constituents of the urine are not given; but if others are to be added, they are to be reported as those

given in the table. The greatest care should be used in this quantitative work. Remember that the man, who is not conscientious, neat and exact in all his work, is not fit for a chemist nor a physiologist, and all analyses made without due care will be of no value. If one goes through the work in an awkward manner, and then *guesses* that it is about right, such a person should have guessed at first and not disgraced the work which he pretends to do. Every thing should be done with that accuracy required by a *scientific* conscience.

§ 209. SPECIMEN TABLE FOR REPORTING THE RESULTS OF A QUANTITATIVE ANALYSIS.

	C. C.	OUNCES.	SPECIFIC GRAVITY.	TOTAL GRAMS.	TOTAL GRAINS.	GRAMS PER KILO, BODY WEIGHT.	GRAINS PER POUND, BODY WEIGHT.
Urine .....	900	30	1025	922.5	14234	14.2	99.6
Total Solids.....				58.25	898.80	.896	6.285
Urea .....				20	308.6	.31	2.16
Total Phosphoric acid ( $P_2O_5$ ).....				3	46.29	.046	.324
Phosphoric acid com- bined with earthy bases.....				1	15.43	.0154	.108
Phosphoric acid com- bined with alkaline bases.....				2	30.86	.0306	.216
Chlorides (NaCl).....				6.5	100.295	.1	.701
Sulphuric acid.....				2.2	33.946	.034	.237
Uric acid.....				.4	6.172	.006	.043

PRELIMINARY EXAMINATION OF URINE.

§ 210. Before the student begins to analyze specimens of urine for diagnostic purposes, he should become perfectly familiar with the reactions of normal urine. Moreover, it will be necessary for him to be able to recognize those substances which may be present in the urine from accidental causes, also to prepare and study both the normal and abnormal constituents of the urine. The student will find it to his advantage to add sugar and other abnormal constituents to normal urine, and then test for them; in this way, should he study as thoroughly.

as possible every substance which may possibly be present in the urine. If one does this work well, he will have no difficulty in making analyses of the urine of his patients.

#### ACCIDENTAL CONSTITUENTS.

§ 211. (1) Examine under the microscope all of the most common starches; as wheat, corn, potato, rice, arrow-root, tapioca, and sago.

(2) Also examine hair, cotton and woolen fibres, bits of feathers, pieces of pine shavings and striated muscular fibre.

(3) Take some saliva from the mouth and examine under the microscope for epithelial scales and salivary corpuscles.

#### BEHAVIOR OF NORMAL URINE WITH ORDINARY REAGENTS.

§ 212. (1) Heat some normal urine in a test tube; if it be strongly acid, no change occurs; if it be but feebly acid, calcium phosphate will be precipitated, and may be redissolved by the addition of a drop of nitric acid.

(2) Heat some normal urine with nitric, hydrochloric, or acetic acid; observe that a peculiar odor is given off, and that the color of the urine becomes darker.

(3) To some normal urine add ammonium hydrate, when ammonio-magnesium and calcium phosphates will be precipitated. Allow the precipitate to subside and then examine it microscopically, when pennate or stellate crystals of triple phosphates will be observed. This deposit is soluble in acetic and the mineral acids.

(4) Render normal urine alkaline by the addition of either sodium or potassium hydrate; an amorphous precipitate of the phosphates of calcium and magnesium will fall, and will be found soluble in acetic and the mineral acids.

(5) Add silver nitrate to some urine acidified with nitric acid, when a precipitate of silver chloride forms. This precipitate is amorphous, and insoluble in nitric acid, soluble in ammonium hydrate (see page 253).

(6) To some urine acidified with hydrochloric acid add barium chloride, when an amorphous precipitate of barium sulphate falls. This precipitate is insoluble in acids (see p. 246).

(7) To normal urine add either uranium acetate or ferric chloride, when a yellowish-white precipitate of the phosphate of uranium or iron forms; either of these will be found insoluble in acetic acid, soluble in hydrochloric acid.

(8) Add to normal urine, oxalic acid or ammonium oxalate, when calcium oxalate will be precipitated (see page 258).

(9) To normal urine add absolute alcohol or chloroform, when a faint cloudiness is produced, either immediately or after standing for some time. This precipitate consists of a kind of albumen normal to the urine (see page 278) and disappears on the addition of water.

(10) The addition of mercuric nitrate to urine produces a precipitate. On the addition of the first few drops of the mercury solution, a precipitate forms and soon redissolves; while on farther addition of mercuric nitrate, a permanent precipitate forms. As the mercury solution first falls into the urine, it unites with the urea forming a precipitate which is immediately decomposed by the chlorides present, forming mercuric chloride. As soon as all the chlorides have been taken up then any farther precipitate formed by the combination of mercury and urea remains undissolved.

(11) Strong tartaric acid solution produces a cloudiness which disappears on the addition of water.

(12) Fresh blood added to warm urine is at first coagulated, then the hæmatin is dissolved from the coagulum by the free acid and colors the urine.

(13) To from thirty to forty c. c. of fresh urine add from eight to twelve drops of tincture of indigo which has been decolorized by hydrogen persulphide. The mixture remains colorless, but is colored on the addition of a few drops of a solution of ferrous sulphate. Both of these reactions fail if a small amount of sulphurous acid be previously added to the urine. From this Schönbein concluded that urine contains traces of hydrogen peroxide.

(14) If urine be heated with starch paste to 60° or 70°, the starch is completely dissolved and grape sugar is formed. If filtered normal urine be treated with from two to three vol-

umes of a 90 per cent. solution of tartaric acid, one obtains a precipitate whose aqueous solution converts starch into sugar.

#### UREA.

§ 213. (1) Prepare crystals of pure urea from the urine (see page 209).

(2) Prepare and study the crystals of nitrate of urea, as obtained from the urine (see page 211).

(3) Obtain the crystal of oxalate of urea from either the urine or from an aqueous solution of urea which has been prepared artificially (see page 211).

#### URIC ACID.

§ 214. (1) Prepare uric acid from human urine and study the forms of the crystals and their solubility in various reagents (see page 222).

(2) Prepare uric acid from either the urine of serpents or from guano (see page 222).

(3) Prepare crystals of alloxan and of nitrate of urea from uric acid (see page 223).

(4) Make the murexid test with some uric acid (see page 224).

(5) Prepare allantoin from uric acid (see page 224).

(6) Prepare and study the acid urates of sodium, potassium, ammonium and calcium (see pages 226 and 227).

#### HIPPURIC ACID.

(1) Prepare hippuric acid from the urine of the horse (see pages 231 and 232).

(2) Take a dose of benzoic acid at night and test the urine passed on rising next morning for hippuric acid (see page 233); or eat greengages and collect the urine passed during the next twenty-four hours and examine it for hippuric acid (see page 234).

#### PHOSPHATES.

§ 215. (1) To some normal urine, add ammonium hydrate; allow the precipitate, which forms, to subside and examine it under the microscope, when stellate or pennate crystals of ammonio-magnesium phosphate will be observed (see page 239).

(2) Set some normal urine aside until the urea gradually

decomposes and the urine becomes alkaline, then examine the deposit under the microscope and observe the prismatic crystals of ammonio-magnesium phosphate (see page 239).

(3) To some normal urine, add potassium or sodium hydrate sufficient to produce an alkaline reaction, when an amorphous precipitate of the phosphates of calcium and magnesium will be thrown down and will be found soluble in acetic and the mineral acids (see pages 239 and 240).

(4) Prepare crystals of the acid phosphate of calcium (see page 240).

(5) Separate the earthy from the alkaline phosphates and precipitate the phosphoric acid of the latter as ammonio-magnesium phosphate (see page 241).

(6) Obtain sodium phosphate,  $\text{Na}_2\text{HPO}_4$ , from the urine (see page 241).

(7.) Prepare crystals of the acid phosphate of sodium from the urine. Also prepare the same artificially (see page 241).

#### SULPHATES.

§ 216. (1) To normal urine, acidified with hydrochloric acid, add barium chloride, when a white, amorphous precipitate of barium sulphate will be thrown down and will be found insoluble in acids (see page 246).

#### CYSTIN.

§ 217. (1) Examine a prepared specimen of cystin under the microscope, studying its crystalline form.

#### CHLORIDES.

§ 218. (1) To some normal urine, acidified with nitric acid, add a few drops of a solution of silver nitrate. Silver chloride is precipitated and should be tested as recommended on page 253.

(2) Prepare crystals of sodium chloride from the urine (see pages 253 and 254).

#### OXALATES.

§ 219. (1) Prepare and study the crystals of calcium oxalate (see page 258).

(2) Set some normal urine aside and examine it from day to day, and note the length of time elapsing between the emission of the urine and the appearance of the crystals of calcium oxalate.

## XANTHIN.

§ 220. (1) Prepare xanthin from muscular tissue according to the method of Stædeler (see pages 268 and 269).

(2) Prepare the precipitates of xanthin with silver nitrate (see page 267).

## GUANIN.

§ 221. (1) Prepare guanin from Peruvian guano (see page 272).

## ALBUMEN.

§ 222. (1) Obtain egg-albumen by beating the whites of eggs with a glass rod, then adding an equal volume of water and filtering through cloth. This albumen is by no means pure, but may be used for the purpose of becoming familiar with the reactions of albuminous urine.

(2) To normal urine, add some of the albumen, prepared as above, and apply the heat and nitric acid test. The student should add the albumen in various proportions and become acquainted with the limits of the reaction. (For the method of applying the tests for albumen, see pages 276 and 277).

## BLOOD.

§ 223. (1) Take a drop of blood from the finger, place it on a glass slide, add a drop of urine, cover with a thin glass, and examine under the microscope.

(2) Obtain a greater quantity of blood from a vein or from a cat or dog, and add it in varying proportions to the urine, and then apply the tests. Examine under the microscope, also by means of the spectroscope, and also apply Heller's test for blood pigment (see pages 278 and 279). Also test for albumen in the urine which contains blood.

## PUS.

§ 224. (1) Obtain pus from some suppurating wound, and examine it under the microscope. The addition of dilute acetic acid to the pus corpuscle will bring out from one to five generally three, nuclei. This test with acetic acid is seldom of any value in the identification of pus in the urine; consequently the student must become perfectly familiar with the appearance of the pus corpuscle. It should be added to the urine in varying proportions, the urine set aside for a while in order to allow



the pus to subside, and then examined under the microscope. It is quite necessary that the student should become expert in the detection of minute traces of pus. Urine containing pus is always albuminous; this is necessarily true; because, the liquor puris contains albumen; but it must be remembered that the quantity of albumen due traces of pus may not be detected. The test for pus by means of the microscope is much more delicate than the test for albumen. Consequently, if no albumen be found by the ordinary test, this is not proof sufficient of the absence of pus. This question will often arise, is there more albumen in a specimen of urine than can be accounted for by the pus or blood present? This can only be answered from the experience and judgment of the analyst. Consequently, the student should add various quantities of pus and blood to normal urine, then compare the abundance of the corpuscles in the deposit with the bulk of albumen thrown down by heat and nitric acid.

#### EPITHELIUM.

§ 225. (1) Kill a cat or a dog, remove the urinary organs and examine the epithelium from the various parts.

#### SUGAR.

§ 226. (1) Dissolve some grape sugar in water (if grape sugar cannot be secured, a substitute may be obtained by dissolving cane sugar in water, acidifying the solution strongly with either hydrochloric or sulphuric acid, and boiling for a few minutes. This solution, when neutralized, readily reduces the copper of Fehling's solution) and apply all of the tests given for sugar (see page 295 et seq.). Then add the solution of grape-sugar to normal urine and apply all of the tests for sugar ascertaining the delicacy of each.

(2) To normal urine, add a little sugar and much albumen and test for the former (see page 296).

#### INDIGOGEN.

§ 227. (1) Prepare indigo from the urine of the horse according to the method of Jaffe (see page 287).

(2) Prepare pure indigo-blue from the indigo of commerce (see page 289).

## UROBILIN.

§ 228. (1) Prepare urobilin from the highly colored urine of a fever patient.

## CHOLESTERIN.

§ 229. (1) Prepare cholesterolin from human gall-stones (see page 53).

(2) Apply the various tests given for cholesterolin on page 54.

## BILE.

§ 230. (1) Dilute some ox-bile (obtained from the slaughter-house) with an equal volume of water, filter and apply Pettenkoffer's test for bile-acids (see page 41). To various dilutions of the bile with water, apply the same test.

(2) To urine, add ox-bile in various proportions and apply Pettenkoffer's test.

(3) To some human, or dog-bile apply Gmelin's test for bile-pigment (see page 55).

(4) To urine containing bile, apply Hoppe-Seyler's modification of Gmelin's test (see page 55).

(5) To the urine of a jaundiced patient, apply the following modification of Gmelin's test: Warm 100 c. c. of the urine, and render it feebly alkaline with barium hydrate. Collect the precipitate, which forms, upon a filter, and dry it. Place a small piece of the dried precipitate in a clean porcelain dish and add a drop of nitrous (fuming nitric) acid, when the series of colors of Gmelin's test will be developed.

(6) To some normal urine, add ox-bile and apply the modification of Pettenkoffer's test as given on page 42.

## TYROSIN AND LEUCIN.

§ 231. (1) Prepare tyrosin and leucin from horn or hair as directed on pages 138 and 140.

(2) To normal urine, add tyrosin and leucin. Concentrate the urine on the water-bath; allow the syrup to cool, and examine it with the microscope. Tyrosin will be found crystallized in needles, which are readily soluble in ammonium hydrate. The leucin appears in brownish discs or globules.

## KREATIN AND KREATININ.

§ 232. (1) Prepare kreatin as given on page 162, and study the form and solubility of the crystals.

(2) Prepare kreatinin from kreatin (see page 163).

(3) Obtain kreatinin from the urine according to the method of Neubauer (see pages 164 and 165).

## INOSIT.

§ 233. (1) Prepare inosit from muscle as recommended on page 169.

## OIL.

§ 234. (1) To some urine, add a drop of milk, or of an emulsion, shake the urine with ether; allow the ethereal layer, which contains the oil, to separate. By means of a pipette, place a few drops of the ether upon a glass slide. Allow the ether to evaporate; add a drop of water to the residue and examine under the microscope for oil globules. These must not be confounded with air bubbles.

## EXAMINATION OF URINE SUSPECTED TO BE ABNORMAL.

§ 235. Collect the urine for the twenty-four hours, mix and measure it. Ascertain the specific gravity and reaction. Set a portion aside in a clean glass vessel (better, a conical one) and allow the deposit to subside for microscopical examination, as given in the following tables (A), (B) and (C). Filter another portion and test the clear filtrate according to table (D).

## MICROSCOPICAL EXAMINATION OF URINARY DEPOSITS.

Allow the urine to stand in a glass vessel undisturbed for some time; then by means of a small pipette or dipping rod, take a drop from the bottom of the vessel; place the drop on a glass slide; cover with a thin glass and examine under a microscope which magnifies from 300 to 500 diameters. The objects seen under the microscope may be either crystallized, amorphous, or anatomical. The same substance may appear at one time in crystals, and at another in the amorphous form, and may thus indicate different pathological results; consequently, the following tables are given:

## (A). CRYSTALS ARE FOUND IN THE DEPOSIT.

Name.	Reaction of the urine in which the deposit occurs.	Form, or forms of the crystals.	Chemical Tests.	Pathological Indications.
Ammonio-magnesium phosphate.	Ammoniacal.	(1). Stellate or Pennate.  (2). Prismatic.	Soluble in acetic acid.  Soluble in acetic acid.	The stellate and pennate forms have no pathological import. They can result only from the immediate addition of ammonia to the urine, and if present, they indicate the addition, either intentional or accidental, of ammonia to the urine after emission (see p. 238). The prismatic form of the triple phosphates indicates that the urine has gradually become ammoniacal from the decomposition of urea. This decomposition may have taken place in the body, which occurs in retention and in inflammation of the bladder. If the decomposition has taken place outside of the body, the crystals will, of course, be indicative of no pathological condition (see pages 200, 201, 202, 238).
Acid phosphate of calcium.	Acid.	Needle-shaped, prismatic, and rhombic.	The needle-shaped and rhombic varieties are to be distinguished from uric acid by the absence of coloring matter, and from both uric and hippuric acids by the ready solubility of the phosphate in hydrochloric acid.	The appearance of these crystals in the urine is probably due to an excess of acids over the bases in the blood. This salt not unfrequently is deposited in the kidney matter, and from both uric and bladder, forming calculi. Hassall has made a study of this substance and finds that deposits of crystallized phosphate of calcium are of frequent occurrence

<p>Oxalate of calcium.</p>	<p>Acid, rarely neutral, or alkaline.</p>	<p>Octohedrons, diamonds, dumb-bells, and discs.</p>	<p>The prismatic form can not be distinguished by optical examination from the corresponding form of the triple phosphate; but the triple phosphate occurs as a deposit only in ammoniacal urine, while this form of calcium phosphate is deposited only in acid urine.</p>	<p>Insoluble in acetic acid and alkalis, soluble in hydrochloric acid.</p>	<p>Insoluble in dilute acids, soluble in alkalis. Collected either by filtration or decantation, washed with alcohol and apply the <i>murexid</i> test (see p. 224).</p>	<p>If oxalate of calcium be found in the urine, within 48 hours after emission, it shows that there is an excess of oxalic acid in the system. This may arise from the kind of food, or from imperfect oxidation. Oxalates frequently form calculi, and more frequently lead to structural disease of the kidney. For further particulars concerning the pathology of calcium oxalate, (see p. 261 et seq.).</p> <p>Free uric acid may be deposited from either of the following causes: (1) the urine may be unduly acid, the stronger acids taking up the bases and setting the uric acid free; (2) there may be an absolute excess of uric acid formed, so that the normal amount of bases is not sufficient to take up all the acid; (3) the proportion of alkaline bases may be abnormally small. If a deposit of uric acid be due to the first and third of these causes, the treatment should consist in the administration of alkalis; if due to the second cause, acid tonics and other</p>
<p>Uric acid.</p>	<p>Acid, rarely neutral, or alkaline.</p>	<p>In a great variety of forms, all soluble in alkalis. Collected of which are the crystals either by filtration or decantation, washed with alcohol and apply the <i>murexid</i> test (see p. 224).</p>	<p>The prismatic form can not be distinguished by optical examination from the corresponding form of the triple phosphate; but the triple phosphate occurs as a deposit only in ammoniacal urine, while this form of calcium phosphate is deposited only in acid urine.</p>	<p>Insoluble in acetic acid and alkalis, soluble in hydrochloric acid.</p>	<p>Insoluble in dilute acids, soluble in alkalis. Collected either by filtration or decantation, washed with alcohol and apply the <i>murexid</i> test (see p. 224).</p>	<p>If oxalate of calcium be found in the urine, within 48 hours after emission, it shows that there is an excess of oxalic acid in the system. This may arise from the kind of food, or from imperfect oxidation. Oxalates frequently form calculi, and more frequently lead to structural disease of the kidney. For further particulars concerning the pathology of calcium oxalate, (see p. 261 et seq.).</p> <p>Free uric acid may be deposited from either of the following causes: (1) the urine may be unduly acid, the stronger acids taking up the bases and setting the uric acid free; (2) there may be an absolute excess of uric acid formed, so that the normal amount of bases is not sufficient to take up all the acid; (3) the proportion of alkaline bases may be abnormally small. If a deposit of uric acid be due to the first and third of these causes, the treatment should consist in the administration of alkalis; if due to the second cause, acid tonics and other</p>

## (A). CRYSTALS ARE FOUND IN THE DEPOSIT.—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form, or forms of the crystals.	Chemical Test.	Pathological Indications.
Urates.	Acid, the urate of ammonium is not unfrequently found in ammoniacal urine.	Urates are seldom in crystals; the urates of ammonium and calcium form needles often arranged in bundles and balls.	Disappear on the application of heat. Soluble in caustic potash and soda; are decomposed on the addition of an acid with the formation of crystals of free uric acid; collect the deposit by either filtration or decantation, wash with a little alcohol, remove to a clean porcelain dish and apply the murexid test (see p. 224).	oxidizing agents should be used (see p. 229, et seq.). Urates are the most common constituents of urinary deposits. They vary in color, from white to crimson; the higher the color, the more serious the indication. An occasional deposit of urates may appear from very trivial causes, as from a change in diet, in the amount of exercise taken, and in the temperature. A deposit of urates occurring in acute inflammatory diseases is an indication for the better, showing that so much of the poison has been eliminated. But a constantly recurring deposit of urates is to be regarded as indicative of some disease of the heart, liver, lungs, or spleen. (See p. 230, et seq.).
Carbonate of calcium.	Neutral, alkaline or feebly acid.	Dumb-bells and discs.	Soluble in acetic acid with effervescence. The dumb-bells and discs of the carbonate are distinguished from those of calcium oxalate by the insolubility of the latter in acetic acid.	This substance is seldom found in the urine of man; but is a constant constituent of the urine of some herbivorous animals. When present in human urine, it is due either to the addition of some carbonate to the urine after emission, or to the decomposition of the urea either in the body, or after emission. The decomposition of the urea yields

Tyrosin.	Acid, neutral or alkaline.	In needles often arranged in bundles.	Freely soluble in ammoniac hydrate. The crystals of tyrosin resemble those of calcium sulphate, but the latter are insoluble in ammonia. The discs and balls of leucicin resemble urates from which they are distinguished by the weak refractive power of the leucicin. They may also be mistaken for oil globules, but the leucicin is insoluble in ether.	carbonate of ammonium, the carbonic acid of which unites with the calcium. The tyrosin and leucicin formed during pancreatic digestion are normally broken up in the liver into urea and uric acid. Any condition of the liver which prevents its normal action in this respect will allow the leucicin and tyrosin to pass on unchanged into the general circulation and appear in the urine (see p. 138).
Leucin.	Acid, neutral or alkaline.	In brownish discs or globules.		
Cystin.	Acid, neutral or alkaline.	Six-sided plates.	Freely soluble in ammonia, insoluble in carbonate of ammonium; soluble in the mineral acids and in oxalic acid, insoluble in acetic and tartaric acids.	The presence of cystin in the urine is due to the imperfect oxidation of the organic sulphur containing constituents of the food. The quantity of cystin excreted in a case of cystinuria varies with the kind of food and with the amount of oxygen which the patient receives. Nitro-muriatic acid and other oxidizing agents are indicated (see p. 251, et seq.). Denotes an imperfect degree of oxidation of the nitrogenous constituents of food and tissue. Xanthin is especially abundant in the blood and urine in leucocytæmia (see p. 269).
Xanthin.	Acid.	In small oval crystals.	Soluble in ammonia; also soluble in strong sulphuric acid, from which it is not precipitated on dilution and thus distinguished from uric acid.	
Cholesterin.	Acid, alkaline or neutral.	In large rhombic plates, clear and thin, and with a characteristic	Soluble in boiling alcohol, from which it crystallizes on cooling. Cholesterin is sometimes found in the urine	Cholesterin forms the principal constituent of biliary calculi; it has been found in the urine in cases of obstruction of the bile-duct, and in fatty de-

## (A). CRYSTALS ARE FOUND IN THE DEPOSIT.—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form, or forms of the crystals.	Chemical Tests.	Pathological Indications.
Calcium sulphate.		<p>teristic notch in one corner.</p> <p>In fine needles resembling tyrosin.</p>	<p>in globules resembling oil, when it should be collected and dissolved in boiling alcohol, and allowed to crystallize.</p> <p>The needles of calcium sulphate dissolve on the addition of an excess of water. They are distinguished from those of tyrosin by the insolubility of the sulphate in ammonia.</p> <p>Easily dissolves on the application of heat.</p>	<p>generation of the kidney.</p> <p>Crystals of calcium sulphate have been found spontaneously deposited in human urine in a single case (Valentiner, Med. Centralbl. S. 913). These crystals may be obtained from the urine of the horse. (See p. 246).</p> <p>A deposit of hippuric acid indicates an excess of this constituent, and is generally due to the kind of food.</p>
Hippuric acid.	Acid.	In needles or rhombic prisms.		



## (B.) AMORPHOUS SUBSTANCES ARE IN THE DEPOSIT.

Name.	Reaction of the urine in which the deposit occurs.	Chemical Tests.	Pathological Indications.
Phosphates of calcium and magnesium.	Alkaline.	Soluble in acetic acid, insoluble in alkalis; distinguished from amorphous urates by the fact that the phosphates do not disappear on the application of heat, and by the failure to obtain crystals of free uric acid by the addition of acetic acid to the phosphates, also by the failure of the deposit of phosphates to respond to the murexid test; distinguished from amorphous oxalates by the insolubility of the latter in acetic acid.	If the deposit consists wholly of the amorphous phosphates of lime and magnesium, the urine is alkaline from a fixed alkali. This deposit often occurs in the urine soon after a meal, and is then due to the excess of alkalis in the food. However, if such a deposit constantly appears in the urine, it indicates an excess of bases over the acids in the blood. Urine constantly alkaline from a fixed alkali denotes a low state of vitality. In the treatment of these cases, two objects may be kept in view. These are (1) to relieve as speedily as possible any irritation of the bladder, and (2) to increase the vitality of the patient, and in this way to remove the cause of alkalinity (see page 243).
Calcium Oxalate.	Acid, rarely neutral or alkaline.	Insoluble in acetic acid, soluble in hydrochloric acid; distinguished from amorphous phosphates by the solubility of the latter in acetic acid; distinguished from amorphous urates by the solubility of the urates in alkalis and on being heated, also by the failure of the oxalates to respond to the murexid test and	Amorphous oxalates are very seldom observed; they may arise either from the addition of oxalic acid to the urine after emission, or from the administration of large quantities of oxalic acid (see page 260).

## (B.) AMORPHOUS SUBSTANCES ARE IN THE DEPOSIT—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Chemical Tests.	Pathological Indications.
Urates.	Acid, the urate of ammonium is not unfrequently found in ammoniacal urine.	<p>to yield crystals of free uric acid on being treated with acetic acid.</p> <p>Determine the presence of urates as in (A). It now remains to determine the base with which the uric acid is combined; collect the urates upon a filter; place some of this collected deposit upon a piece of platinum and heat in the flame of a Bunsen burner or spirit lamp; (1) it communicates an intense yellow color to the flame, <i>sodium urate</i>; (2) it imparts a violet color to the flame, <i>potassium urate</i>. Remove another portion of the collected deposit to a clean porcelain dish, add a little potassium hydrate and then heat; if the vapor of ammonia is given off, and colors a red litmus paper held over the dish, the deposit contains <i>ammonium urate</i>.</p>	<p>Urates are the most common constituents of urinary deposits. They vary in color from white to crimson: the higher the color the more serious the indication. An occasional deposit of urates may appear from very trivial causes: as from a change in diet, in the amount of exercise taken and in the temperature. A deposit of urates occurring in acute inflammatory diseases is an indication for the better, showing that so much of the poison has been eliminated. But a constantly-recurring deposit of urates is to be regarded as indicative of some disease of the heart, liver, lungs or spleen. (See page 230, et seq.)</p>

## (C) ANATOMICAL OR FORMED CONSTITUENTS ARE FOUND.

Name.	Reaction of the urine in which the deposit occurs.	Form.	Means of Detecting.	Pathological Indications.
Pus.	Acid, neutral or alkaline.	In corpuscles.	By microscopic appearance.	The presence of pus shows undue inflammation. If the pus be from the bladder, it will generally contain much mucus, giving the deposit aropy consistency, and the urine will frequently be alkaline from a volatile alkali. In suppurative cystitis, the greater part of the pus and mucus will be passed after the water; while in pyelitis the pus will be distributed through the urine, which will generally be of an acid reaction. In urethritis, the first urine passed will contain all, or the greater part of the pus. (See page 279).
Blood.	Acid, alkaline or neutral	In corpuscles, or dis- * integrated.	By the microscopical detection of the corpuscles, or by spectroscopical examination, or by Heller's test for blood pigments. See page 245.	The presence of blood in the urine may be due either to a physiological (as in menstruation) or to a pathological hæmorrhage. If there be clots large enough to be visible to the unaided eye, the blood must have passed into the urine below the secreting structures. If from the bladder the clots will often be quite large, and may obstruct the passage through the urethra. If the coagulation has taken place in the ureters, the shape and size of the clots will so indicate. When from the pelvis of the kidney, the coagula are much smaller than those from the bladder, and may preserve the shape of the calices.

## (C) ANATOMICAL OR FORMED CONSTITUENTS ARE FOUND—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form.	Means of Detecting.	Pathological Indications.
Epithelium.	Acid, alkaline or neutral.	Pavement and columnar.	By microscopical examination.	<p>If from the substance of the kidney only, the clots will be microscopic in size, having been formed in the tubules, and the urine will generally have a smoky tint. (See p. 278).</p> <p>(1) Epithelium from the uriniferous tubules consists of small circular pieces, with a large nucleus in the center.</p> <p>(2) Pieces from the pelvis of the kidney are triangular and polyhedral, thicker than, but not so large as, those from the bladder and vagina.</p> <p>(3) From the ureters, the pieces are conical or triangular, with a nucleus near the base; they are smaller than those from the urethra.</p> <p>(4) From the bladder, large and small spheroidal pieces, the small ones more nearly circular, the larger ones are polyhedral, and often elongated at one corner.</p> <p>(5) From the urethra (male) columnar, conical, with a nucleus near the base.</p> <p>(6) Vaginal epithelium is pavement, polyhedral, more oval than those from the bladder. If the pieces of epithelium are normal in appearance but unduly abundant, only an excessive desquamation is indicated;</p>

but if they contain globules of oil, or give the amyloid reaction (see page 285) the organ is undergoing degeneration.

(1) *Hyaline* casts are smooth, structureless, and may be detected by the addition of a solution of iodine in iodide of potash, or of a dilute solution of carmine, when they will be stained yellow or red, respectively. These casts are formed by the coagulation of albumen in the nriniferous tubules, and simply indicate albuminuria.

(2) *Epithelial* casts are cylinders or pipes, formed by the removal of the epithelia of the tubules in mass. They are caused by inflammation of the mucous membrane of the kidney.

(3) *Granular* casts consist of masses of aborted epithelia, differing from the epithelial casts in the fact that the individual cells are not fully developed. They indicate a more inflamed state of the mucous membrane of the kidney than is indicated by the epithelial casts.

(4) *Bloody* casts consist of coagulated albumen with blood corpuscles entangled, and are formed in hæmaturia.

(5) *Waxy* casts have the appearance presented by melting a piece of wax, dropping it upon a glassslide, and allowing it to cool. They are formed by an abnormal secretion from the kidney, and indicate a more serious condition of this organ than is indicated by any other form of casts.

An excess of mucus shows undue irrita-

Casts.	Acid, neutral or alkaline.	<p data-bbox="186 1024 217 1176">In cylinders.</p> <p data-bbox="186 641 290 924">Examine the deposit under a microscope which presents a clear field.</p>
Mucus.	Acid, neutral or In corpuscles, and in	There is no difference.

## (C) ANATOMICAL OR FORMED CONSTITUENTS ARE FOUND—Continued.

Name.	Reaction of the urine in which the deposit occurs.	Form.	Means of Detecting.	Pathological Indications.
Spermatozoa.	alkaline. Acid, neutral, or alkaline.	a gelatinous mass.  About .045 of a millimeter long, with one extremity enlarged, flattened, triangular, and termed the 'head.' The other extremity, the 'tail,' is long and slender. In globules, either floating through the fluid, or contained in casts and epithelium.	between mucous and pus corpuscles. Mucus is gelatinous andropy. Examine under a microscope which magnifies at least 300 diameters.	tion of some part of the urinary tract. The source of the mucus is to be ascertained as given under pus. Their occasional appearance is of little or no importance, but their continued or frequent presence shows that there is something either mental or physical which unduly excites the glands.
Oil.	Acid, neutral, or alkaline.		Agitate some urine in a test tube with ether; remove some of the ether and place on a glass slide, allow the ether to evaporate, add a drop of water to the residue, cover with a thin glass and examine under the microscope for oil globules.	Oil is often accidentally present in the urine from bottles not thoroughly cleansed, use of catheter, etc. Casts and epithelium containing oil globules are found in fatty degeneration of the kidney.
Sarcina Ventriculi, (Merismopodia punctata).	Acid, neutral, or alkaline.	Appear as small cubical moving masses. They often appear greenish or brownish.	By microscopical examination, they are distinguished from vibrios by the difference in shape.	These algæ may be so abundant in the urine as to cause a visible grayish deposit. They have been found in catarrh of the bladder.

Torulæ.	Acid.	In small oval spores, which may be found single or attached to each other.	By microscopical examination. Sometimes one sporule will be observed just budding out from another.	These fungi appear only in urine which contains sugar, and show that the sugar is undergoing the process of fermentation.
Penicillium.	Acid.	In sporules, and interlacing branches, which are seen to be composed of separate parts.	By microscopical examination. The separate sporules cannot be distinguished from the torulæ; but the thallus of the well-developed penicillium differs from that of the sugar fungus.	These fungi may appear in any acid urine on standing. It was formerly supposed that albugen was necessary for their development, but this theory has been proved to be false. However, they are more likely to occur in urine which contains either albumen or sugar than in any other.
Vibrios.	Acid, neutral, or alkaline.	Small moving threads or filaments.	By microscopical examination. These little bodies differ greatly in length and in other respects.	They arise from the decomposition of organic matter, and may be formed in the bladder in cystitis.

## (D) EXAMINATION OF THE CLEAR FILTRATE.

Name.	Reaction of the urine in which the substance occurs.	Influence of the constituent upon the physical properties of the urine.	Chemical Tests.	Pathological Indications.
Urea.	Generally strongly acid when there is an excess of urea; and feebly acid, neutral, or alkaline when there is a deficiency of urea.	When there is an excess of urea, the specific gravity is high and the color is deep; when there is a deficiency of urea the specific gravity is low and the color is light.	The absolute amount of urea excreted in a given time can be ascertained only by a quantitative analysis. (See p. 303). Whether the urea is in excess or deficient in proportion to the water may be ascertained by the following tests: <i>Test for Excess.</i> —Place a drop of urine on a glass slide and add a drop of nitric acid; leave in a cool place. If within five minutes an abundant crop of crystals of nitrate of urea appear, the urea is in excess. <i>For Deficiency.</i> —Evaporate some urine to half its bulk. Take a drop and proceed as in excess. If no crystals are formed in five minutes, there is deficiency of urea.	There is an excess of urea in most febrile diseases and in diabetes. The increase of urea in fever is generally in exact proportion with the temperature of the body. (See p. 219).
Chlorides.	Acid, neutral, or alkaline.	A deficiency of chlorides may appear in either pale or highly colored urine; or the spe-	Whether there be a deficiency of chlorides or not can be ascertained only by a quantitative analysis. (See p. 306).	With regard to chlorides, we may say that an excretion of less than one gram for the 24 hours is abnormal. In pneumonia, typhus fever, acute rheumatism and erysipelas,



Phosphates.	Acid, neutral or alkaline.	<p>cific gravity may be either high or low.</p> <p>No characteristic effect upon the physical properties of the urine.</p>	<p>Whether there be an excess or deficiency of phosphates can be ascertained only by a quantitative analysis. (See p. 308).</p>	<p>common salt is diminished and frequently is not found at all in the urine. (See p. 256 et seq.).</p> <p>There is an excess of phosphates in the urine in inflammatory diseases of the nervous system and in rickets and osteomalacia. There is deficiency in indigestion and in structural diseases of the kidney. (See page 244).</p>
Sulphates.	Urine with an excess of sulphates is generally acid.	<p>Urine containing an excess of sulphates is of high specific gravity.</p>	<p>A quantitative estimation must be made of the 24 hours' urine. (See p. 307).</p>	<p>An increase of sulphates arises either from an excess of sulphates in the food, or from the use of medicinal sulphates. Otherwise, sulphates vary with the degree of oxidation and are increased in febrile diseases and decreased in skin diseases and in all cases of imperfect oxidation. (See p. 248, et seq.).</p> <p>Ammonium carbonate results from the decomposition of urea.</p>
Carbonates.	Generally ammoniacal.	<p>There is always a deposit in urine which contains carbonates if any appreciable quantity.</p>	<p>Effervescence is produced on the addition of acetic acid, and if the gas be passed into lime water, the latter will be rendered turbid by the precipitation of carbonate of calcium.</p>	
Leucin and tyrosin.	Acid, neutral or alkaline.	<p>Urine containing these constituents is frequently colored with bile.</p>	<p>Concentrate the urine and examine under the microscope for crystals as given in (A).</p>	<p>Leucin and tyrosin occur in the urine in severe structural diseases of the liver—see table (A).</p>
Cystin.	Acid, neutral or alkaline.	<p>Fresh urine containing cystin has a sweet briar odor; when decomposition sets in, hydro-</p>	<p>Cystin is precipitated from alkaline solutions by acetic acid, and from acid solutions by ammonium carbonate. The precipitate is collected, dissolved in ammoniacal</p>	<p>Cystin results from the imperfect oxidation of the sulphur which exists in organic combination in the food, as in eggs and beef. Its presence is presented by the use of</p>

## (D) EXAMINATION OF THE CLEAR FILTRATE—Continued.

Name.	Reaction of the urine in which the substance occurs.	Influence of the constituent upon the physical properties of the urine.	Chemical Tests.	Pathological Indications.
Blood.	Acid, neutral or alkaline.	<p>sulphuric acid gas is given off.</p> <p>Urine containing much blood is more or less colored. It may be blood-red or smoky or even black.</p> <p>Dark-red or greenish.</p>	<p>hydrate and the solution is concentrated, when six-sided crystals of cystin will be obtained.</p> <p>To the solution apply Heller's test for blood pigment. (See p. 279).</p> <p>Also examine with the spectroscope.</p>	<p>oxidizing agents. See table (A).</p> <p>See table (C). If the blood be from the kidney, the urine will be smoky or dark; while if the blood be from the bladder, or the urethra, the color of the urine will be red.</p>
Bile.	Generally acid, though it may be neutral or alkaline.	Highly-colored.	<p>Apply Pettenkoffer's test for bile-acids. (See p. 41), and Gmelin's test for bile-pigments. (See p. 55).</p> <p>Examine with the spectroscope. (See p. 293).</p>	<p>Bile appears in the urine in obstruction of the bile-duct, and in excessive formation of bile.</p>
Urobilin.	Acid, neutral or alkaline.	The urine may be highly colored or pale.	<p>To from 4 to 6 c. c. of strong hydrochloric acid in a test tube, add from 20 to 40 drops of the urine and heat gently, when, if indigogen be in excess, a violet or blue color will be developed. (See p. 289).</p> <p>Albumen is coagulated both by heat and nitric acid. Each of these</p>	<p>Urobilin is increased in all febrile diseases and causes the high color of the urine of fever patients.</p> <p>Indigogen is in excess in the urine in cases of obstruction of the intestines, also in catarrh of the intestines, and in the urine first passed after a cholera collapse.</p>
Albumen.	Acid, neutral or alkaline.	Urine containing much albumen		<p>The albumen may come from the bladder, as in cystitis, from the pel-</p>

Sugar.	Generally acid.	is generally pale and frequently of low specific gravity.	should be applied, first separately and then together" (See p. 276).	vis of the kidney, as in pyelitis, and from the serum of the blood directly as in structural diseases of the kidney. (See p. 278, et seq.).
Sugar.	Generally acid.	Urine containing much sugar is increased in amount, pale and of high specific gravity.	Apply Trommer's test. (See p. 295), and Moore's test. (See p. 297). If these are not sufficiently satisfactory, add the fermentation test. (See p. 298).	Diabetes mellitus has been known to follow upon (1) injury to the head, with or without fracture of the skull; (2) clot in the pons varoli; (3) softening at the base of the brain; (4) disease of the sympathetic nerve; (5) excessive brain work; (6) uterine disease; (7) disordered digestion; (8) exposure to cold, etc. (Harley).
Paralactic Acid.	Acid.	Seldom found save in diabetic urine.	Concentrate the urine to a small volume on the water-bath; while yet warm transfer to a large flask and add 95 per cent. alcohol, boil and continue the addition of alcohol as long as a considerable precipitate remains. Allow to stand for 24 hours. Pour off the clear alcoholic solution and evaporate it to a syrup. Add dilute sulphuric acid and shake with ether. Spontaneous evaporation of the ethereal extract leaves a brown strongly acid syrup, which on being diluted with water forms oily drops. Remove the coloring matters by precipitation first with normal then with basic lead acetate. Remove the lead with hydrogen sulphide and evaporate the filtrate on the water-bath. Add water and again	Found in diabetes and <i>probably</i> in some forms of rheumatism.

## (D) EXAMINATION OF THE CLEAR FILTRATE—Continued.

Name.	Reaction of the urine in which the substance occurs.	Influence of the constituent upon the physical properties of the urine.	Chemical Tests.	Pathological Indications.
Hydrogen Sulphide.	Neutral, acid or alkaline.	Arises from decomposition of sulphur containing organic bodies.	<p>evaporate on the bath. The residue is paralactic acid and the various salts may be prepared as directed. (See p. 173).</p> <p>Place some of the urine in a small beaker or flask, and cover with paper moistened with lead acetate and gently warm when the paper will be blackened.</p>	An excess of sulphur containing organic substances.

[§ 238.]

**Examination of Urine.**

For..... at the request of  
Dr. ....

**Physical and Chemical Characters.**

Total quantity for 24 hours.....:

Color..... Odor.....

Reaction..... Sp. Gr. ....

Deposit, quantity and general appearance.....

Urea..... Urates.....

Phosphates..... Sugar.....

Albumen.....

**Microscopical Examination.**

Crystals .....

Anatomical elements .....

Other morphological elements.....

## Pathological Indications.

Dated.....

---

### EXAMINATION OF URINARY CONCRETIONS.

• § 239. *Preliminary.*—Gravel should be coarsely crushed and the particles examined with a microscope. In some instances the crystalline form may be recognized. Calculi should be divided into halves by means of a jeweler's fine saw. The nucleus, as the most important part of the calculus, should be carefully examined. It may consist of one of the urinary constituents or of a foreign body. In rare instances the center of the calculus is a cavity. In this case, the nucleus was mucus which has dried up, leaving the cavity. Calculi may be composed solely of one constituent or of two or more arranged in layers. After the stone has been cut into halves, and one of the cut surfaces polished upon a glass plate, the different layers of the several constituents are easily recognized. Particles of each layer should be separately detached with a pen-knife and subjected to chemical analysis. The powdered gravel, the dust from the saw in cutting the stone, or particles from the different layers should be examined as follows:

Heat to redness on a piece of platinum and observe (1) whether the whole or any part remains unburnt; (2) the color of the flame if there be any visible; (3) the odor, if any.

(A) If the greater part or the whole has been driven off by the heat, special tests should be made for each of the following substances: uric acid, ammonium urate, cystin, xanthin and protein substances\*.

---

\*The urostealith concretions of Heller are simply phosphatic calculi with a fatty or waxy foreign substance as a nucleus. Recently Professor Maclean, of Michigan University, removed a phosphatic stone with a nucleus of chewing gum. The patient, a boy, acknowledged having introduced the gum some years before.

(a) *Uric Acid and Ammonium Urate*.—To some of the powder in a clean porcelain dish apply the murexid test (see page 224). To distinguish between free uric acid and ammonium urate, boil some of the powder with water and filter while hot. The urate will be dissolved while free uric acid would remain upon the filter. Heat some of the powder with potassium hydrate when ammonium, if present, would be evolved and may be recognized by its odor and by its vapor coloring moist red litmus paper blue.

(b) *Cystin*.—Dissolve the powder in ammonium hydrate and allow to evaporate spontaneously on a glass slide, when cystin, if present, will be deposited in hexagonal plates. Dissolve another portion in hydrochloric acid, from which cystin is deposited in needles arranged in groups. Cystin burns with a bluish flame, and gives off the odor of burning sulphur and fat. Calculi of cystin have a fatty or waxy lustre, are soft, and have a soapy feel.

(c) *Xanthin*.—Dissolve in\* nitric acid and evaporate, the yellow residue, if xanthin be present, is not colored by ammonia (means of distinguishing from uric acid), but forms a reddish-yellow solution when treated with potassium hydrate. Calculi of xanthin are very rare. They take a waxy lustre on being rubbed and consist of layers which are easily separated.

(d) *Protein Substances (Fibrin, Blood-Clots, Etc.)*.—Burn with a yellow flame and give off the odor of burning feathers. They dissolve in potassium hydrate, from which they may be precipitated by acids. Protein calculi are very rare.

(B) If any portion remain incombustible, it may contain one or more of the following substances: potassium, sodium or calcium urate, calcium oxalate, calcium carbonate, calcium phosphate and ammonio-magnesium phosphate.

(a) *Urates*.—To some of the powdered gravel or stone apply the murexid test. To ascertain the base, boil the powder with distilled water and filter while hot. Evaporate the solution, which contains the urates, and ignite the residue. If this residue after ignition colors moist red litmus paper blue, either sodium or potassium or both are present. Test specially for each of these bases. A particle of the residue moist-

ened with a drop of hydrochloric acid and held on a platinum wire in the colorless flame of a Bunsen burner, if sodium be present imparts to the flame a yellow color which is hidden by the blue glass (glass colored with cobalt). Potassium, when present, gives a violet flame, hidden by the sodium flame but not obscured by the blue glass. Dissolve a portion of the ignited residue in hydrochloric acid, render the solution alkaline by the addition of ammonium hydrate, then add sodium hydrogen phosphate, when calcium, if present, will be precipitated.

(b) *Calcium Oxalate*.—Heat some of the powder, which may at first blacken from the presence of organic matter, but subsequently it becomes white, but does not fuse. Dissolve the residue in dilute hydrochloric acid and to this solution add ammonium hydrate and oxalic acid, when calcium will be precipitated as an oxalate. Calcium oxalate is insoluble in alkalis and acetic acid, and dissolves in hydrochloric acid without effervescence.

(c) *Calcium Carbonate*.—Dissolves in acetic and hydrochloric acids with effervescence (means of distinguishing from calcium oxalate). Test for the calcium as given under calcium oxalate. Calculi of calcium carbonate or calculi containing this substance are very rarely found in man; but they are common in the herbivorous animals.

(d) *Phosphatic Calculi*.—Basic calcium phosphate and ammonio-magnesium phosphate are found mixed in the same stone. Phosphatic calculi when heated fuse and form an enamel-like mass. They are soluble in both hydrochloric and acetic acids without effervescence. A precipitate is produced in this solution by the addition of ammonium hydrate. To determine what bases are present proceed as follows: Dissolve the fused mass in dilute hydrochloric acid, reprecipitate with ammonium hydrate, redissolve with a few drops of acetic acid, avoiding an excess; now add ammonium oxalate, when calcium, if present, will be precipitated as an oxalate. Remove the precipitate by filtration and saturate the filtrate with ammonium hydrate, when the ammonio-magnesium phosphate will be precipitated.



## DETECTION OF MEDICINAL SUBSTANCES IN THE URINE.

§ 240. This is a branch of the analysis of urine to which no great attention has been given, and it may, at first, seem unnecessary to discuss it here; but let us consider its importance. It is well known that some of the most common medicines often produce strange, and at times, injurious effects. This is sometimes due to an accumulation of the medicine in the system; one dose is given, and if it does not produce certain effects in a given time, the physician administers another without knowing whether the first has been either entirely or partially eliminated. I have made quite a number of experiments in this line with iodide and bromide of potassium, and with morphia. In some cases, I have found that after administering a medicinal dose of these substances, they appeared in the urine within less than an hour's time, and disappeared within twenty-four hours; while in other cases they cannot be detected in the urine until the expiration of twenty-four hours. Now suppose that these medicines are given at certain intervals of time to two patients; in one, the substance is rapidly eliminated: in the other, it is unduly retained; the doses are repeated, giving one as much as the other, the system of the first contains only the ordinary dose, while that of the second may contain three or four times the medicinal dose. I have no doubt but the life of many a patient could have been saved from the cumulative action of medicinal poisons by a timely examination of the urine; and this is the only apology I will offer for introducing this subject.

MORPHIA,  $C_{17}H_{19}NO_3H_2O$ .

§ 241. Concentrate the urine to one-tenth its bulk, render it alkaline with ammonia, and shake well with amyllic alcohol. Separate the alcohol and evaporate it to dryness; to a portion of the residue, add two or three drops of concentrated  $H_2SO_4$ , and heat on the water-bath for one hour, then add a drop of  $HNO_3$  which will produce a deep red color if morphia be present. Treat a second portion of the residue with iodic acid

and bisulphide of carbon. The morphia liberates iodine, which colors the bisulphide.

STRYCHNIA,  $C_{22}H_{24}N_2O_2$ .

§ 242. Concentrate the urine to a syrup, render strongly alkaline with KHO, and agitate well with chloroform. Separate the chloroform and evaporate it to dryness on the water-bath; to the residue add strong  $H_2SO_4$  and heat on the water-bath for one hour, then neutralize with sodium carbonate, and render alkaline with KHO; agitate again with chloroform; separate the chloroform and evaporate to dryness in a small porcelain dish on the water-bath; dissolve the residue in a few drops of  $H_2SO_4$ , then slowly move a small crystal of potassium bichromate through this solution; if strychnia be present, the crystal will produce a purple coloration.

VERATRIA,  $C_{32}H_{52}N_2O_8$ .

§ 243. Concentrate the urine to a syrup, render alkaline with KHO, agitate with chloroform, remove the chloroform and evaporate it to dryness on the water-bath; treat the residue with ether, remove the ether and evaporate it to dryness. To a portion of the residue, add a few drops of concentrated  $H_2SO_4$ , and heat on the water-bath, when a crimson color will be produced, if veratria be present. Dissolve the remaining part of the residue in HCl; this solution is colorless, when cold, dark red when warm.

ATROPIA,  $C_{17}H_{23}NO_3$ .

§ 244. Evaporate the urine to dryness on the water-bath, add a few drops of KHO, and agitate with ether; remove the ether, evaporate it to dryness; dissolve the residue in chloroform; remove the chloroform and evaporate it to dryness; dissolve the residue in water, and place a drop of this solution in the eye; if atropia be present the pupil will be dilated.

SANTONIN,  $C_{15}H_{18}O_3$ .

§ 245. Santonin imparts a deep red color to alkaline urine. If the urine, when passed, be of normal reaction, no peculiarity of color will be observed, but upon the addition of an alkali, the

characteristic color will be produced; this color disappears after standing, or after being agitated with oxygen.

#### IODINE AND BROMINE.

§ 246. When iodides or bromides are administered in medicinal doses, they may be detected in the urine, upon the addition of chlorine water and bisulphide of carbon.

#### ARSENIC AND ANTIMONY.

§ 247. Evaporate the urine to dryness; to the residue add fuming nitric acid, and heat on the sand-bath until all the organic matter is destroyed. Dissolve the residue in water strongly acidified with HCl. Treat this solution with  $H_2S$  gas for twenty-four hours; collect and wash the precipitate, and remove it to a porcelain dish or crucible, cover with fuming nitric acid, and heat to dryness. Treat the residue with water, which will dissolve the arsenic but not the antimony. Test the water solution for arsenic by Marsh's test. If the substance be antimony, it can be dissolved in dilute HCl, and precipitated with  $H_2S$  gas, the precipitate having the characteristic orange-red color.

For details of this method for detecting and separating arsenic and antimony, see a paper by the author, in the American Chemist for August, 1875.

#### MERCURY.

§ 248. Evaporate the urine to dryness and destroy the organic matter with nitric acid as given under arsenic and antimony. Mix the residue with sodium carbonate and potassium bichromate, put this into a tube which is opened at one end and has a bulb blown at the other. Shake the mixture into the bulb and heat, keeping the open end of the tube cool. The mercury is vaporized, and collects in small globules upon the upper and cool extremity of the tube.



## ERRATA.

Page 46, for  $C_{27}H_{46}NO_5$  read  $C_{27}H_{43}NO_5$ .

Page 52, for  $NH_4$ ,  $C_2H_4$ ,  $SO_2$ ,  $HO$  read  $NH_3$ ,  $C_2H_2$ ,  $SO_2$ ,  $H_2O$ .

Page 138, for  $C_9H_{16}NO_3$  read  $C_9H_{11}NO_3$ .

Page 10, line 9, for service read surface.

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CHEMICAL  
PHYSIOLOGY AND PATHOLOGY.  
PART II—PLATES.



## EXPLANATION.

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As is shown by the title page, these plates constitute a part of the work on Chemical Physiology and Pathology. When the text was published, the author intended that the plates should appear upon charts; but further consideration of the subject together with advice from many teachers, who are using the text, has led to the presentation of the plates in the present form. It is intended that the plates are to be used especially in connection with the tables for the systematic analysis of urine given in the text (pages 324-340). In these tables are given briefly the conditions and physical appearance of all the crystalline, amorphous and organized deposits which are likely to appear in the urine, and all these deposits are represented in the following plates. All the objects are represented as seen when magnified four hundred diameters; the original drawings having been made by the aid of a Zentmayer's microscope, with his one-fifth inch objective and B eye-piece, and which was found to magnify four hundred diameters.

The student must constantly bear in mind that the microscope is only an aid to the physiological chemist, and in all cases where new, rare or doubtful forms appear, their nature must be determined by chemical tests. The microscope is a valuable help in the analysis of animal and vegetable fluids, but this means of diagnosing objects needs always to be used with caution. For instances so far as the microscopic appearance is concerned, urea, hippuric acid, phosphate of magnesium, phosphate of calcium, and oxalate of urea may be mistaken, one for another. The writer has not unfrequently known tyrosin to be reported as present in a specimen of urine, when the crystals seen were really those of phosphate of magnesium. Such an error as this is of the worst kind. Acting upon the evidence supposed to be furnished by the examination of the urine, the physician, in this case, regards his patient as suffering from a very grave disorder, while in truth a correct analysis of the urine would not indicate any serious abnormality. IN CASES WHERE ANY DOUBT CAN ARISE AS TO THE TRUE NATURE OF THE OBJECTS SEEN UNDER THE MICROSCOPE, APPLY THE CHEMICAL TESTS.

## DESCRIPTION OF CUT.

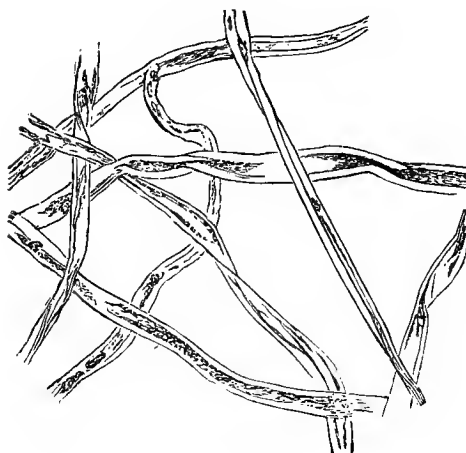
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### THE KÜHNE-STRAUCH APPARATUS FOR THE DETECTION OF AMMONIA IN BLOOD AND OTHER ANIMAL FLUIDS.

The flask *B* serves for the reception of the blood or other fluid to be examined. *A* is a hydrogen generator, *a* is a drying tube filled with pumice stone washed with sulphuric acid. This tube is united by air tight tubes on the one side with the hydrogen generator and on the other with the flask *B*. This flask has a stopper pierced by three openings, through one the tube from *a* passes down beneath the surface of the fluid, through the second passes the glass tube *b* which also extends below the surface of the fluid and the outer end of which can be closed by a piece of rubber and clamp as shown in the figure, through the third opening a tube, which does not extend to the surface of the fluid in *B*, passes to the bottom of the Woulfe bottle *C*. This bottle serves to remove the foam carried over with the gas from *B*. On the other hand the Woulfe bottle is connected with the apparatus *D* which contains Nessler's reagent (a solution of potassium iodide in one of mercuric iodide).

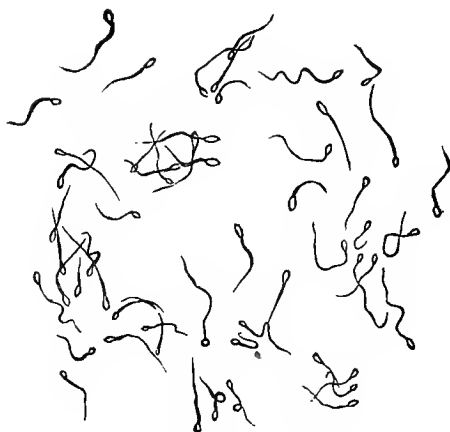
In operating, close the tube *b* with the clamp and make all the connections with the exception of the apparatus *D*. Generate hydrogen in *A* from chemically pure zinc and sulphuric acid. When the apparatus is filled with hydrogen, connect the apparatus *D* containing Nessler's reagent, allow the blood to flow directly from the vein through *b* into *B*, close *b* again and continue the generation of hydrogen. Any free ammonia contained in the blood is carried by the hydrogen gas into *D*. A trace of ammonia produces a reddish-yellow coloration, while more produces a bluish precipitate in the Nessler reagent.

FIG. 1.



Cotton Fibres.

FIG. 2.



Spermatozoa as found in the urine of an insane man addicted to masturbation.



FIG. 3.



Common forms of Uric Acid.

FIG. 4.

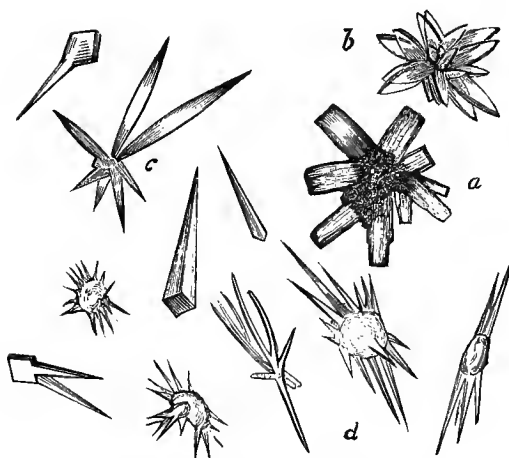


Prismatic form of Uric Acid,



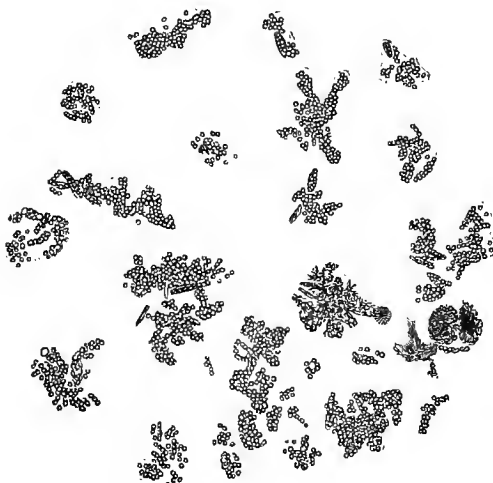


FIG. 5.



Rare forms of Uric Acid (Harley).

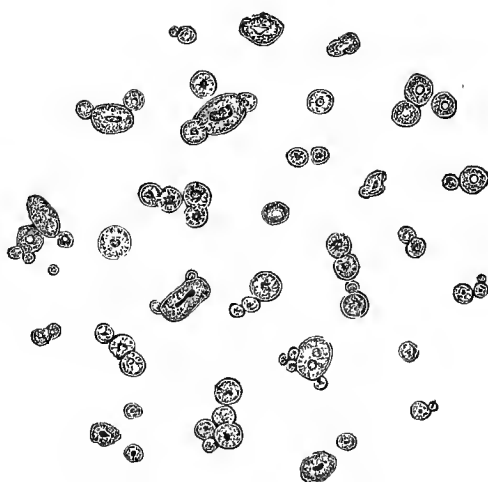
FIG. 6.



Urate of Ammonia.

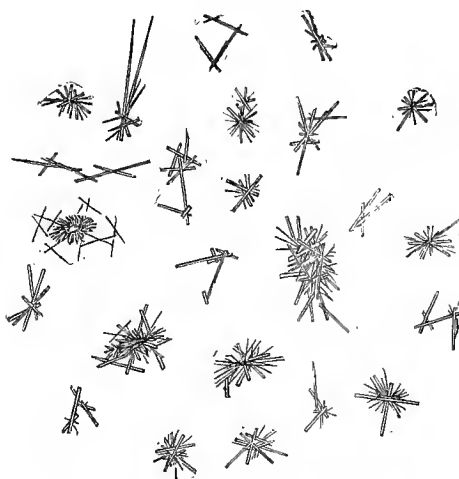


FIG. 7.



Spherules of Urate of Soda.

FIG. 8.



Urate of Soda prepared artificially,

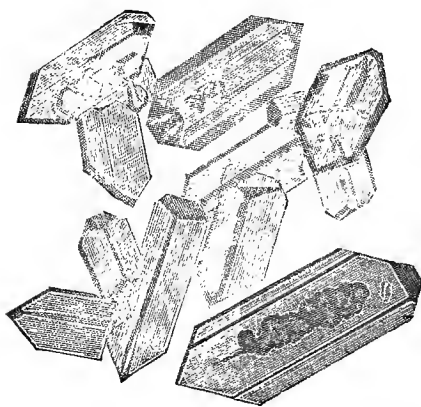


FIG. 9.



Ammonio-Magnesian Phosphate.

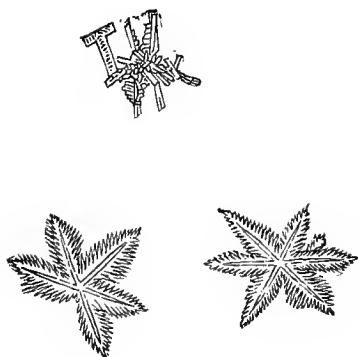
FIG. 10.



Prismatic Ammonio-Magnesian Phosphate.



FIG. 11.



Ammonio-Magnesian Phosphate precipitated by the addition of  
Ammonia to normal urine.

FIG. 12.

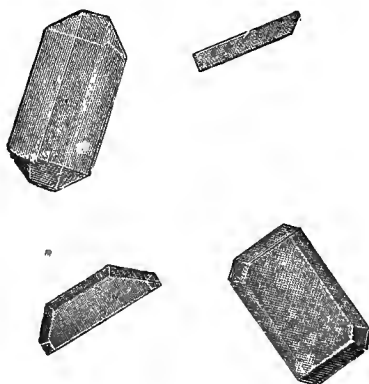


Acid Phosphate of Lime,



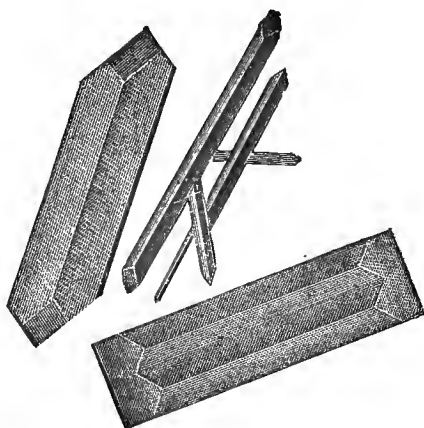


FIG. 13.



Acid Phosphate of Soda.

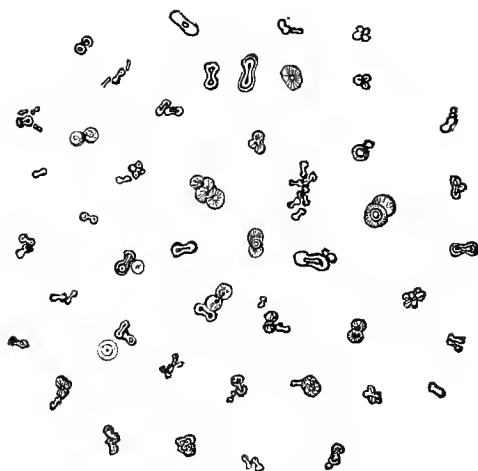
FIG. 14.



Phosphate of Magnesium (Robin and Verdeil).



FIG. 15.



Dumb-Bells of Oxalate of Lime.

FIG. 16.



Different forms of Oxalate of Lime (Harley).



FIG. 17.

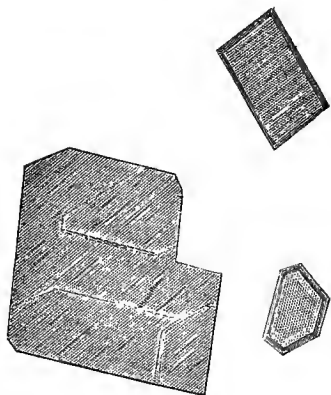
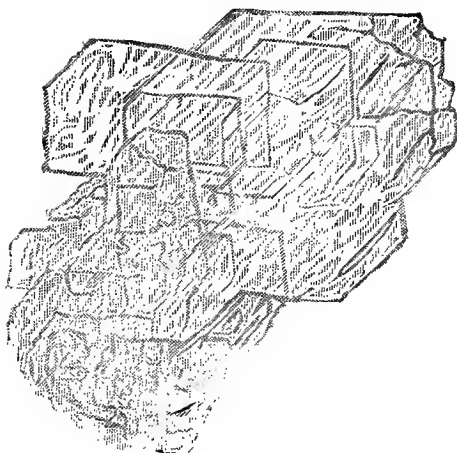


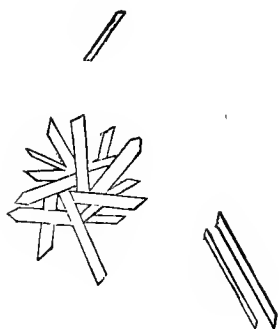
FIG. 18.



Nitrate of Urea (Robin and Verdeil).

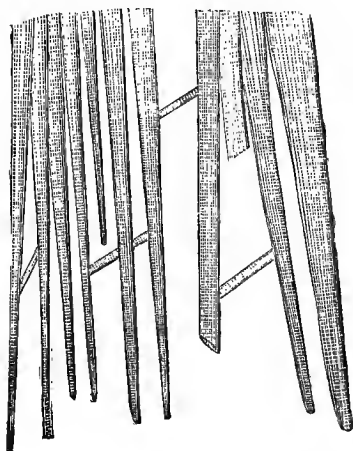


FIG. 19. \*



Oxalate of Urea (Robin and Verdeil).

FIG. 20.

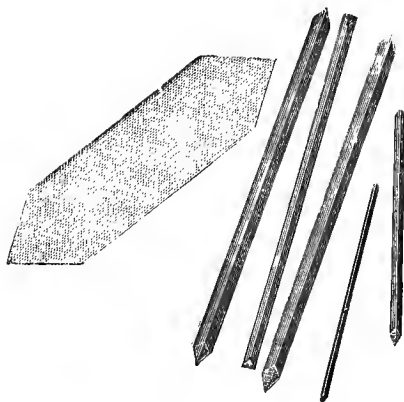


Urea (Robin and Verdeil).





FIG. 21.



Hippuric Acid (Robin and Verdeil).

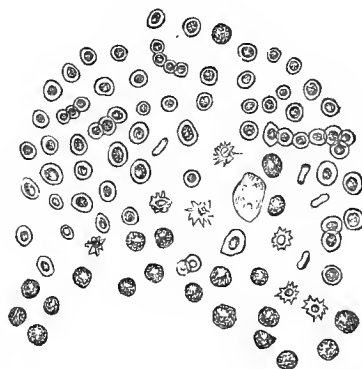
FIG. 22.



Pus; a, before, and b after the addition of dilute acetic acid (Bowman).



FIG. 23.



Blood (Hoffmann and Ultzmann).

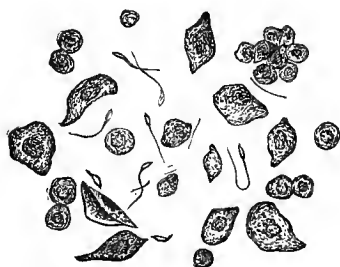
FIG. 24.



Epithelium ; the squamous from the bladder and the columnar from the ureter and urethra (Tyson).

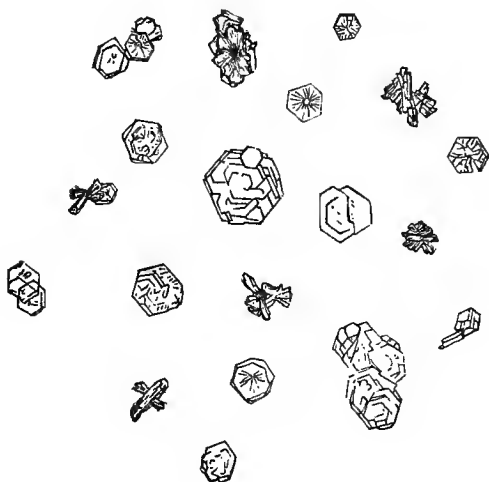


FIG. 25.



Vaginal Epithelium and Spermatozoa taken from the vagina of a little girl upon whom rape had been committed (Beale).

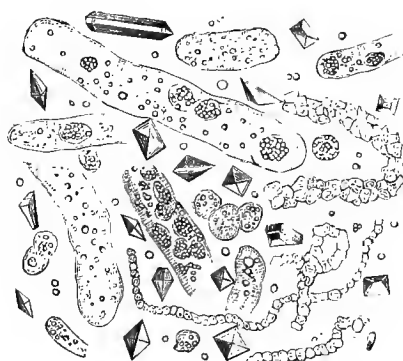
FIG. 26.



Cystin.

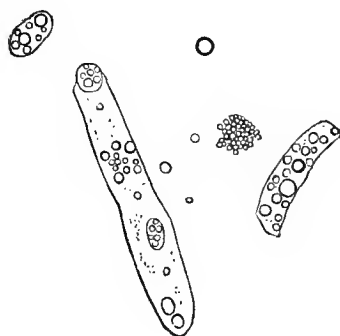


FIG. 27.



Crystals of triple phosphates with prismatic portion defective,  
and granular and oily casts (Beale).

FIG. 28.

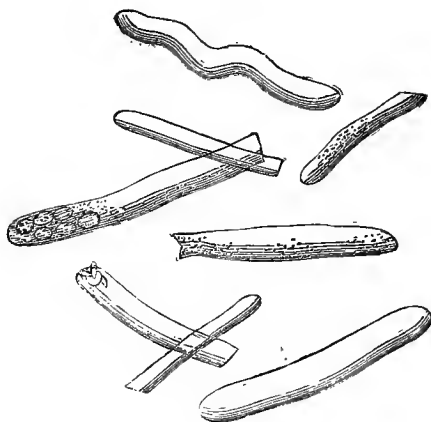


Oily Casts (Tyson).



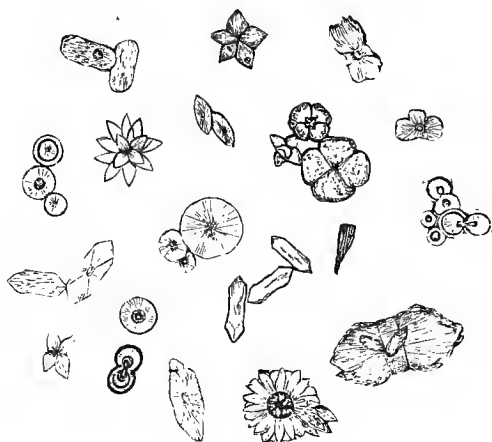


FIG. 29.



Smooth and slightly granular casts (Harley)

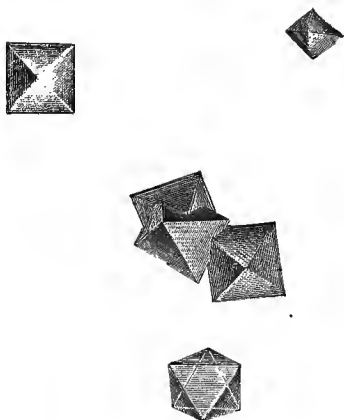
FIG. 30.



Carbonate of Lime.

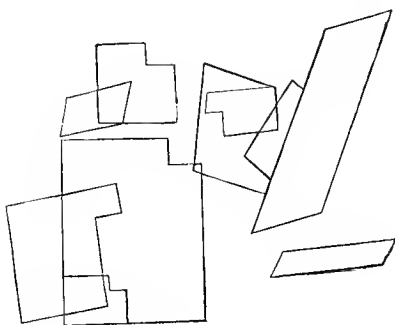


FIG. 31.



Chloride of Sodium.

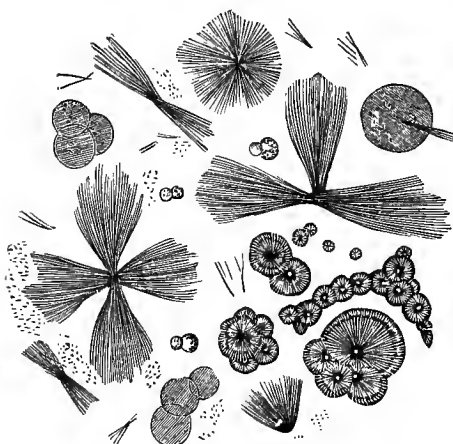
FIG. 32.



Cholesterin (Harley).

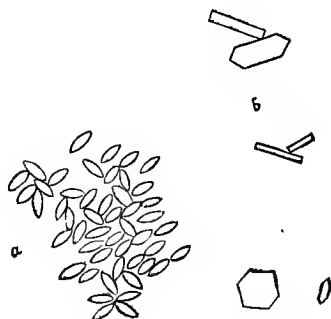


FIG. 33.



Tyrosin Needles and Leucin Spherules (Tyson).

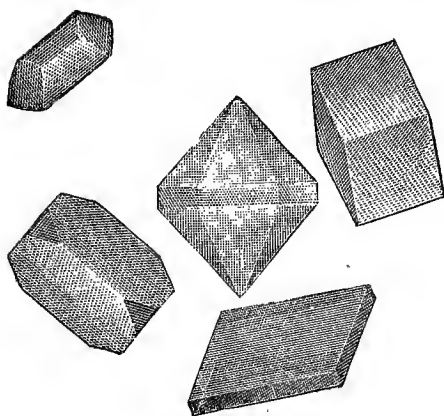
FIG. 34.



Xanthin (Harley).

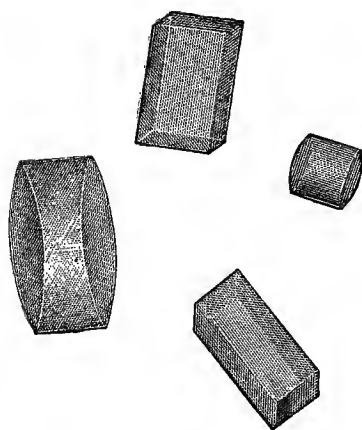


FIG. 35.



Creatin.

FIG. 36.

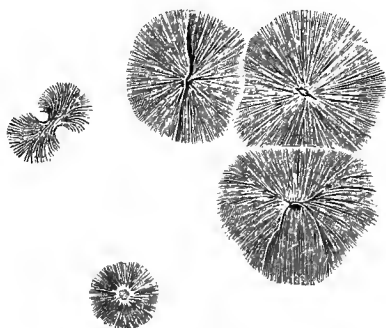


Creatinin.



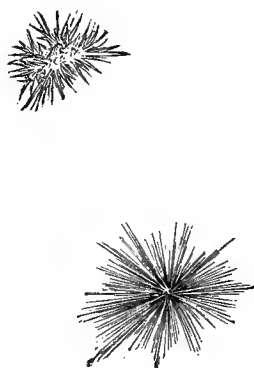


FIG. 37.



**Double Chloride of Creatinin and Zinc (Robin and Verdeil).**

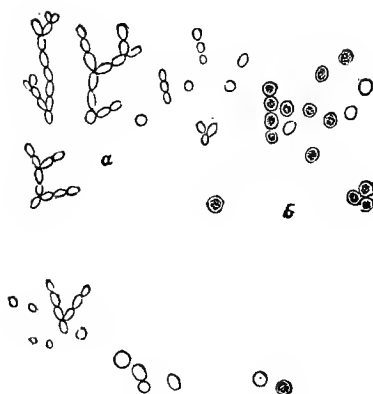
FIG. 38.



**Sulphate of Lime.**

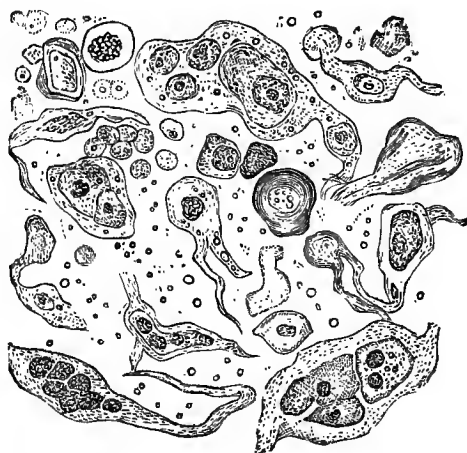


FIG. 39.



a *Torulæ Cerevisiæ* from diabetic urine, b Sporules from baker's yeast (Harley).

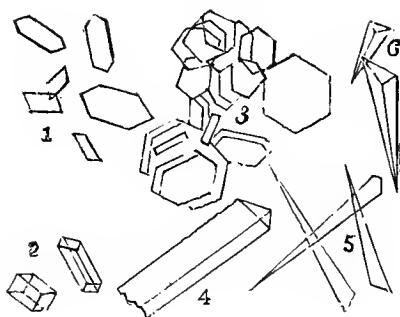
FIG. 40.



Cancer cells from urine in case of Cancer of the Uterus (Beale).



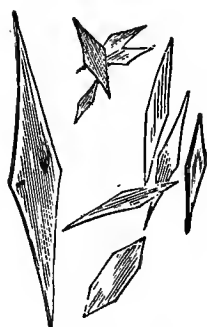
FIG. 41.



Crystals of Hæmoglobin: (1) from blood of rabbit, (2) from blood of hedgehog, (3) from blood of mouse, (4) from blood of cat, (5) from blood of lark (Bojanowsky).



FIG. 42.

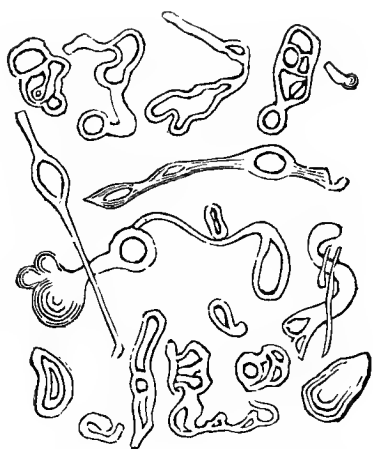


Crystals of Charcot-Neumann (Hofmann). See page 110 of text.





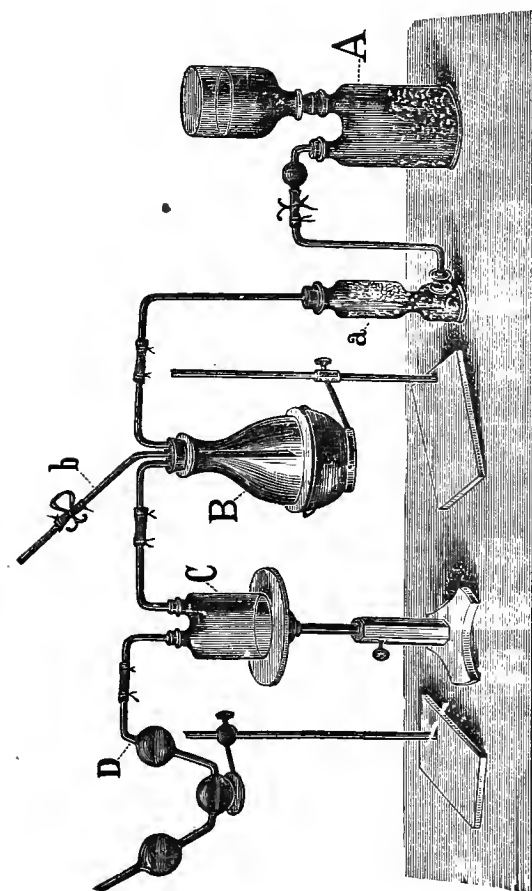
FIG. 43.



Cerebrin (Hofmann) See page 175 of text.



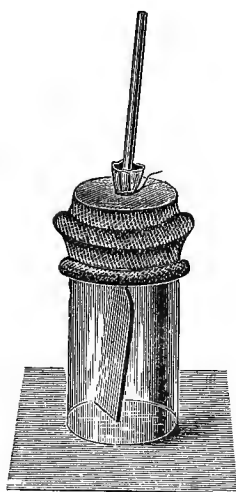
FIG. 44.



Kühne-Strauch Apparatus for the Detection of Ammonia in Blood and Serous Fluids (Görup-Besanez). See explanation page 4, part II.



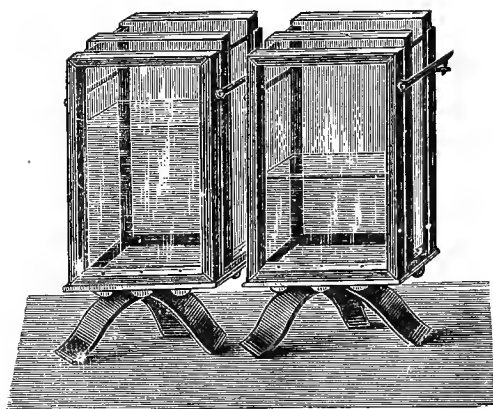
FIG. 45.



Apparatus for the Estimation of Fibrin (Hoppe-Seyler). See page 95 of text.



FIG. 46.

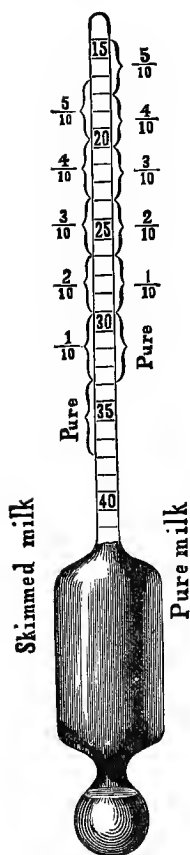


Hæmatinometer (Hoppe Seyler). See page 82 of text.





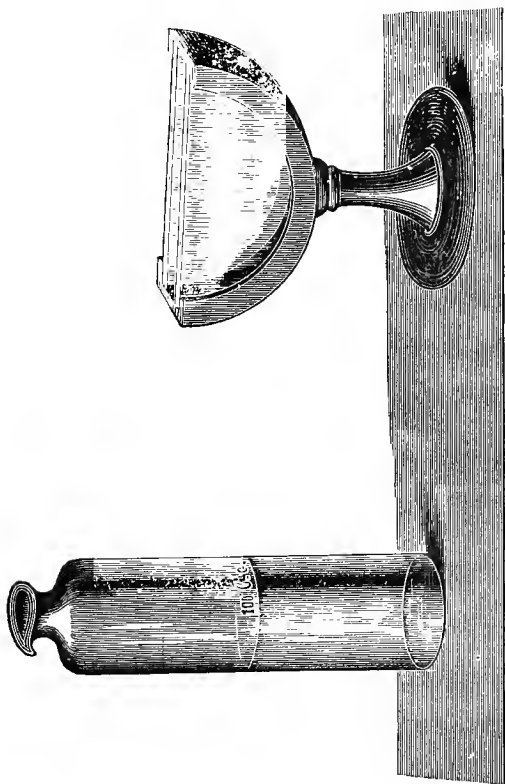
FIG. 47.



Lactodensimeter. See page 130 of text



FIG. 48.



Apparatus for Estimation of Fat in Milk by Vogel's Method  
(Gorup-Besanez). See page 128 of text.









